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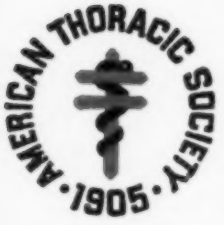

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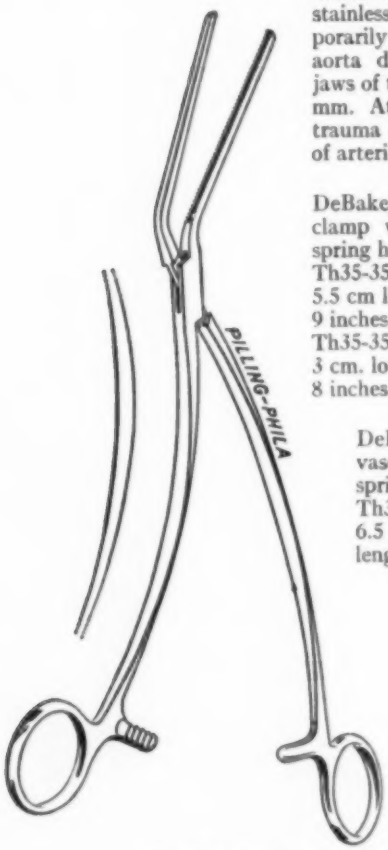

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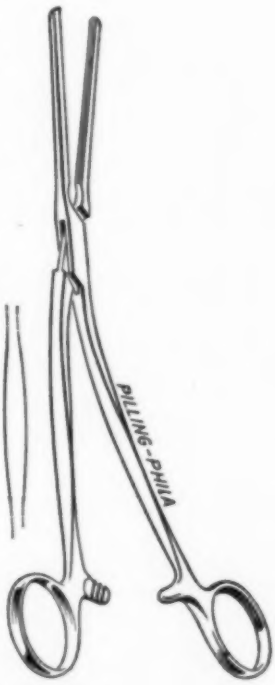

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THE AMERICAN REVIEW OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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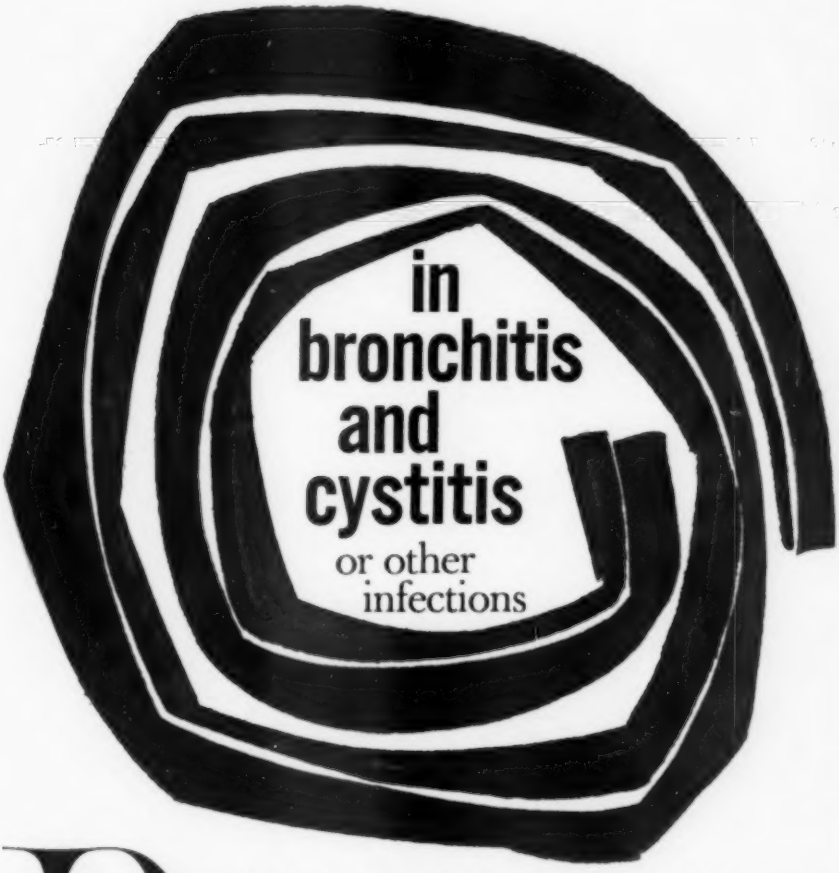

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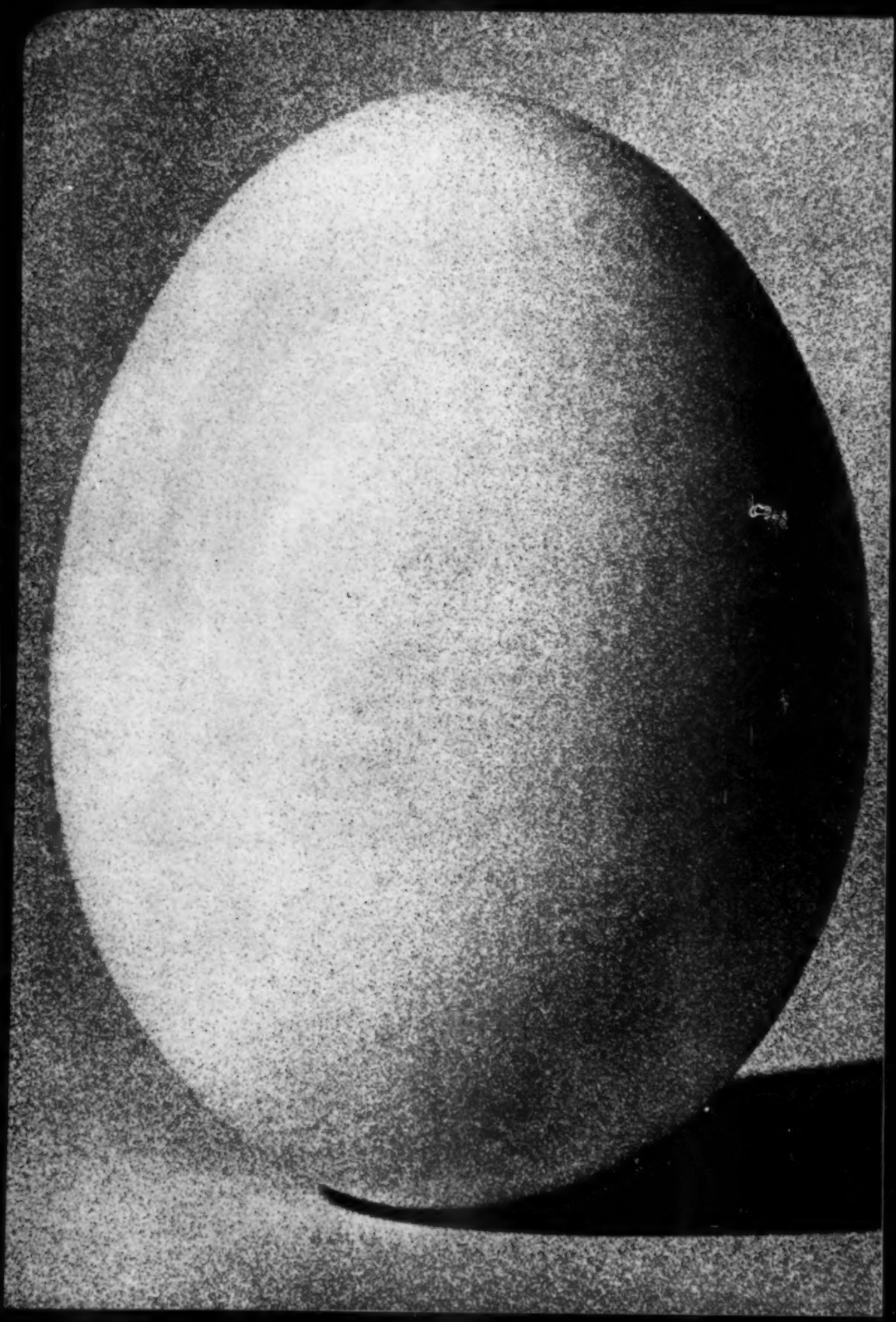

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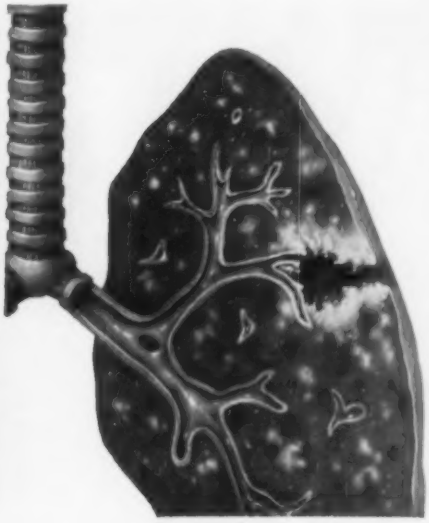

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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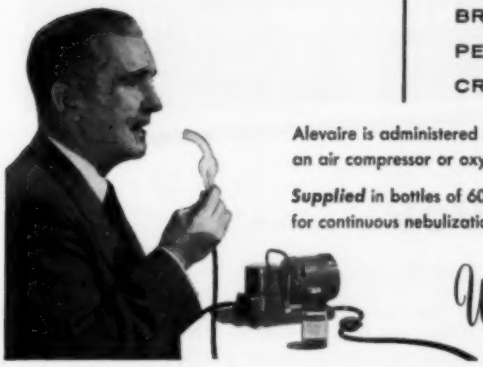

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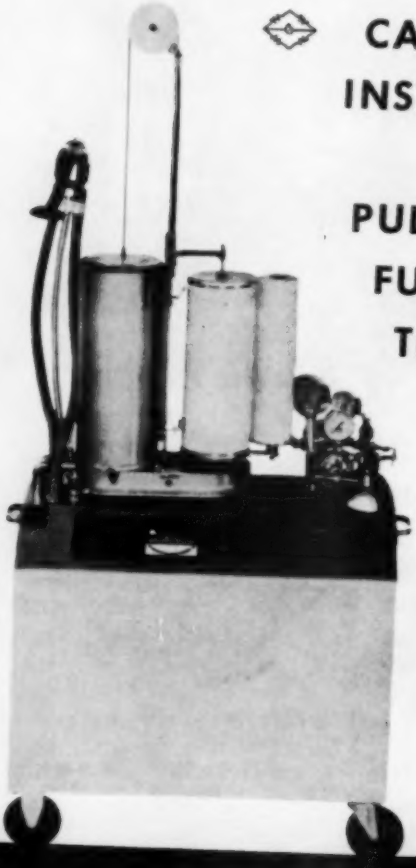

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THE AMERICAN REVIEW
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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 84 | *October 1961* | NUMBER 4

THE NASHVILLE AIR POLLUTION STUDY[1, 2]

I. Sulfur Dioxide and Bronchial Asthma. A Preliminary Report

L. D. ZEIDBERG, RICHARD A. PRINDLE, AND EMANUEL LANDAU

(Received for publication May 31, 1961)

INTRODUCTION

Two of man's greatest discoveries, fire and the wheel, are directly and indirectly responsible for the major part of man-made air pollution today. The early cave dweller must have exposed himself and his family to air pollutants every time he built a fire in his cave, but it was largely a localized situation affecting only a few people. Fire-produced air pollution really became a community problem in the early part of the fourteenth century after the discovery of coal as a fuel. London was among the first communities to experience the effects of coal smoke. There the atmosphere became so intolerable that the burning of coal was prohibited by royal decree. Had the prohibition remained in effect, the smog disaster that occurred some 650 years later in London (1952) might have been avoided, and the lives of the 4,000 to 8,000 persons whose deaths were attributed to that episode (1) might have been spared.

The wheel has influenced the quality of the ambient air somewhat less directly. While man made the wheel turn by his own power, by applied animal strength, or through the use of natural force such as water power, the atmosphere remained unaffected. Once man invented fuel-powered engines to turn his wheels, however, he produced such far-reaching effects on his atmospheric environment that the health and comfort of millions of people in a city like Los Angeles are endangered.

By the early seventeenth century it was quite generally known that the irritant in coal smoke was sulfur dioxide (2). This substance was later suspected of being responsible for the high morbidity and mortality associated with the notorious smog disasters that occurred in Belgium's Meuse Valley in 1930 (3); in Donora, Pennsylvania in 1948 (4); and in London in 1952 (1). In each instance, adverse meteorologic conditions interfered with the dilution and dispersal of pollutant emissions into the atmosphere and produced an unusual concentration of offending substances. The resulting symptomatology in the affected population was characterized chiefly by respiratory distress, but included also eye irritation and lacrimation, headache, nausea, and vomiting. Persons with chronic pulmonary or cardiac disease appeared to be most affected in these smog episodes. The pattern of reaction to intense and prolonged smog was fairly well delineated in these disasters, but what happens to those who are exposed over a period of many years to lesser concentrations of air pollutants, such as sulfur dioxide, is less well known.

Experimental investigations in both man and animals have shown that bronchospasm is produced with the inhalation of even low concentrations of sulfur dioxide (5), and increased pulmonary flow resistance develops (6). When the concentration of the gas is increased, adverse effects progress to increased production of mucus and even to severe inflammatory response. Normal lung function would be modified by any of these reactions (7, 8).

[1] From Vanderbilt University School of Medicine, Nashville, Tennessee; and Division of Air Pollution, U. S. Public Health Service, Department of Health, Education, and Welfare, Washington, D. C.

[2] Presented before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 22, 1961.

Bronchial asthma is characterized by bronchospasm and edema of the bronchial mucosa. Asthmatic patients might be expected to react more violently than others to the irritating effect of sulfur dioxide. In the Donora Study it was observed that the smog affected 87.6 per cent of asthmatic patients compared with 77.2 per cent of cardiac patients and 42.7 per cent of the total population in the Donora area (4). A higher mortality from asthma and bronchitis was found among persons living in an industrial area, compared with those living at some distance from it (9). Bronchial asthma following exposure to sulfur dioxide fumes has been reported by Romanoff (10) and Dowling (11). Both investigators believed the irritant action of the gas to be responsible for the attacks. On the other hand, it has been proposed by Cooke (12) that sulfur dioxide could play the role of an allergenic inhalant and produce an allergic reaction in sensitive individuals. In this belief he has been supported by Urbach and Gottlieb (13) and by Brown and Columbo (14). There is, however, no immunologic proof of the concept.

A rather curious type of chronic asthma has developed during the last decade in Japan (15, 16), and on Okinawa (17, 18). It appears to be definitely associated with atmospheric pollution caused by industrial emissions. In the United States, New Orleans has experienced recurrent high frequencies of asthmatic attacks that have taxed the emergency hospital facilities of that city from time to time. The exact cause of these episodes remains to be determined (19).

From the foregoing, there is an obvious need for more research to fill the gaps in the knowledge concerning the effects of long-term exposure to the usual pollutants in the ambient air over urbanized areas. The Air Pollution Medical Program[a] of the Public Health Service has sponsored and supported such research for several years. The Nashville Air Pollution Study was set up by contract with the Department of Preventive Medicine of Vanderbilt University School of Medicine (20) for the purpose of developing and testing methods of investigating the health effects of air pollutants. Four separate but related studies were undertaken. One of these, the Cardio-Respiratory Disease Study, was designed to observe the effects of air pollution on a group of subjects known to be suffering from chronic cardiac and/or respiratory disease.

Collaborating in these studies was the Air Pollution Engineering Program[a] of the Public Health Service's Robert A. Taft Sanitary Engineering Center. The engineers established a network of 123 sampling stations in a pattern that placed residents of the study area no farther than a half-mile from a sampling site (figure 1). A full description of the objectives and plans of both the medical and engineering aspects of the joint study has been reported previously (21).

The present paper is a preliminary report of observations made on 84 persons with chronic bronchial asthma who comprised approximately two-thirds of the entire Cardio-Respiratory Disease Study Group. A more complete report will be made when the analyses are completed.

Material and Methods

Objectives

1. To determine whether persons with chronic bronchial asthma show variation in the frequency and severity of their attacks depending on variation in their exposure to environmental air pollutants.

2. To determine whether the course of disease in persons with chronic bronchial asthma differs according to their residence in an area of relatively high or low air pollution.

The Study Population

The cardiorespiratory study group consisted of 123 subjects, most of whom were outpatients in the Vanderbilt University Hospital Clinic and a few who were patients of local private physicians. Included in this panel of patients were 49 adults and 35 children with chronic bronchial asthma. The age, sex, and race distribution of these 84 patients is shown in table 1. White and nonwhite were almost equally represented, 51.2 and 48.8 per cent, respectively. Males constituted 52.4 per cent of the group and females, 47.6 per cent; but the relative distributions were not uniform at all ages. One-half of the males as compared with one-fourth of the females were in the age group less than fifteen years old.

The residence of the study group is shown in figures 2 and 3. The adults tended to live closer to the Clinic than the children.

The occupation of the adults is shown in table 2. With the exception of 17 housewives and 8 female private household workers, there is no concentration of occupations and no indication that asthmatic attacks were occupationally provoked in the group under study.

[a] Now part of the Division of Air Pollution, U. S. Public Health Service.

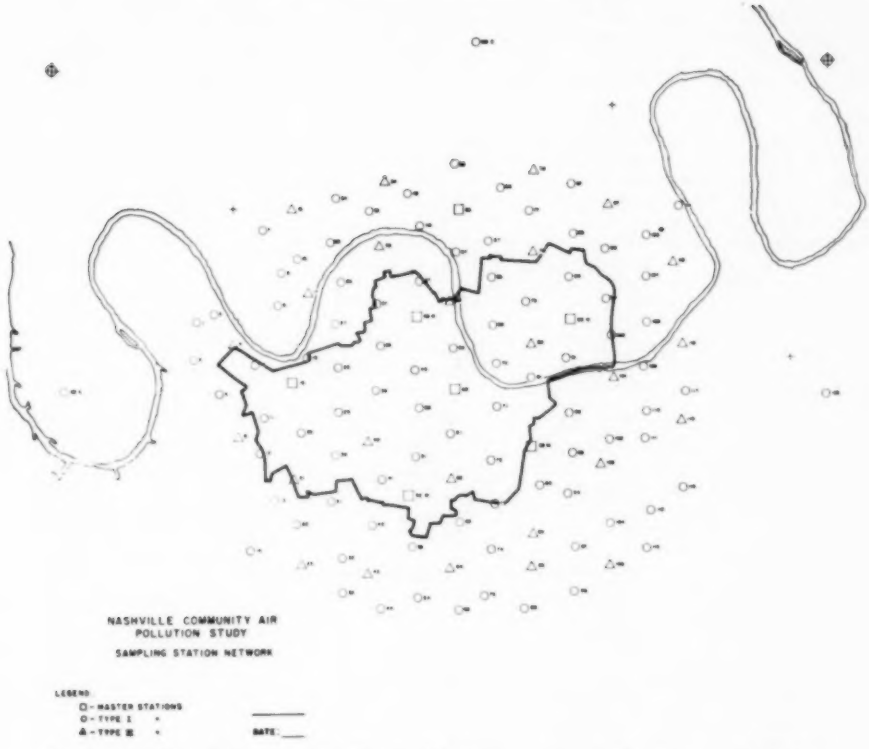

FIG. 1. Sampling station network. The outlined area represents the city boundary lines. The river is the Cumberland.

TABLE 1

DISTRIBUTION OF THE ASTHMATIC PATIENTS IN THE STUDY GROUP BY AGE, SEX, AND RACE
NASHVILLE AIR POLLUTION STUDY

Age Group	Total						White						Nonwhite					
	Total		Male		Female		Total		Male		Female		Total		Male		Female	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
years																		
Total	84	100.0	44	100.0	40	100.0	43	100.0	25	100.0	18	100.0	41	100.0	19	100.0	22	100.0
0–14	34	40.5	24	54.5	10	25.0	20	46.5	13	52.0	7	38.9	14	34.1	11	57.9	3	13.6
15–24	6	7.1	5	11.4	1	2.5	3	7.0	3	12.0	0	—	3	7.3	2	10.5	1	4.5
25–34	6	7.1	1	2.3	5	12.5	4	9.3	1	4.0	3	16.7	2	4.9	0	—	2	9.1
35–44	10	11.9	3	6.8	7	17.5	4	9.3	1	4.0	3	16.7	6	14.6	2	10.5	4	18.2
45–54	15	17.9	5	11.4	10	25.0	3	7.0	2	8.0	1	5.6	12	29.3	3	15.8	9	40.9
55–64	7	8.3	2	4.5	5	12.5	4	9.3	1	4.0	3	16.7	3	7.3	1	5.3	2	9.1
65–74	4	4.8	2	4.5	2	5.0	3	7.0	2	8.0	1	5.6	1	2.4	0	—	1	4.5
75–84	2	2.4	2	4.5	0	—	2	4.7	2	8.0	0	—	0	—	0	—	0	—

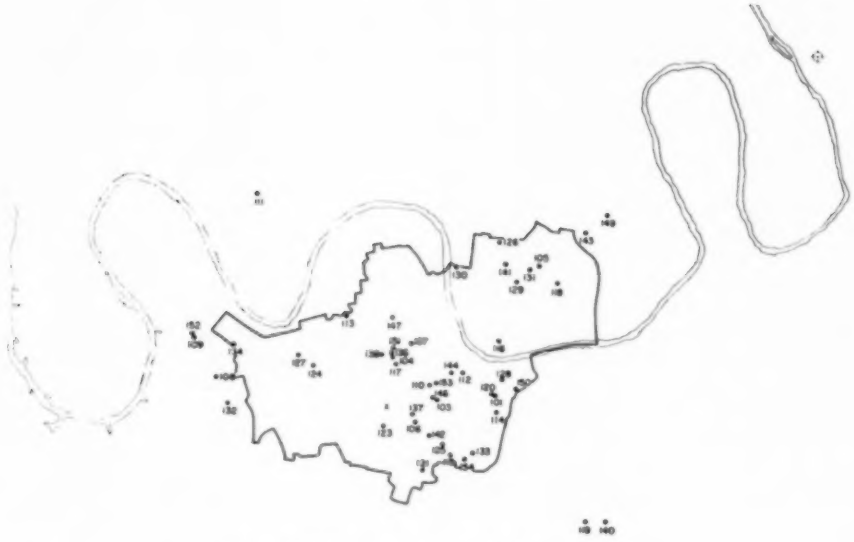

FIG. 2. Residence of adult asthmatic patients.

FIG. 3. Residence of pediatric asthmatic patients.

Plan of the Study

Data concerning the occurrence of asthmatic attacks in panel members were collected through weekly reporting on special forms printed on postal cards (figure 4). The times of onset and end of each attack, the place of occurrence, and medication taken, if any, were to be recorded daily, and the cards were to be mailed weekly to the Study office.

The asthmatic patients selected for the Study panel were briefed concerning the need for their cooperation and the kind of studies to which they would be subjected, but they were never told that the investigations were related to air pollution research. They were deliberately kept in ignorance of the Study's orientation in order to avoid possibly biased reporting.

The panel members were shown a report card, its terms were defined, and they were instructed in the proper notations to be made. They were asked to keep the record daily and to mail the card to the Study office on Monday mornings.

A new card with the next week's dates already written on it was mailed to each panel member at the end of each wek. Enclosed was a reminder to mail the current week's card on Monday. If cards were not received by Wednesday, the delinquent panel members were called on the phone or reminded again by mail if they had no phones. If such reminders failed to produce the completed card, a public health nurse associated with the Study made a house visit to the patient. Most of the patients appeared in the allergy clinic at weekly intervals, and at that time also they were reminded to keep their records current and mail their cards on time. Opportunity was also taken during the clinic visit to make a spot check of the reliability of reporting by asking the patients about their attacks, and then checking the information received by interview against the card reports. In general, reporting was found to be quite reliable. Failure to report was less than 1 per cent finally.

TABLE 2

OCCUPATIONAL CHARACTERISTICS OF THE ADULT ASTHMATIC PATIENTS

NASHVILLE AIR POLLUTION STUDY

Code	Latest Occupational Group	Total	Male	Female
	Total	49	19	30
12	Professional, technical, and kindred workers	2	0	2
13	Managers, officials, and proprietors	2	2	0
31	Clerical and kindred workers	2	2	0
33	Sales workers	1	1	0
41	Craftsmen, foremen, and kindred workers	1	1	0
42	Operatives and kindred workers	3	2	1
51	Private household workers	8	0	8
52	Service workers, except private household	4	3	1
62	Laborers, except farm and mine	1	1	0
98	Housewives	17	0	17
99	Retired	3	3	0
XX	Students	4	3	1
00	Unemployed for other reasons	1	1	0

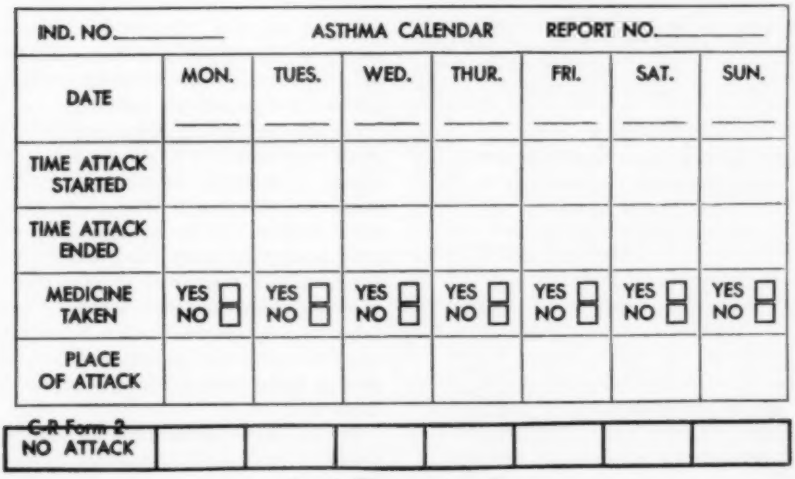

FIG. 4. The report card.

Initially, all panel members had complete histories taken, including histories of allergy and of tobacco use for the adults. A complete physical examination was done, and routine laboratory tests were performed. A chest film was obtained on each patient. In addition, several pulmonary function tests were done. These included the vital capacity, expiratory flow rates, and pulmonary resistance, using the vitalometer, Wright peak flow meter, and Clements interrupter for the respective tests. From the vital capacity, the timed vital capacity, midexpiratory time, midexpiratory flow rate, maximal expiratory flow, and EFR_{50} were calculated. These tests were repeated frequently during the patients' clinic visits. The clinical records dating back to the first clinic admission of each patient were available to the Study staff. Two of the staff (Drs. Sidney Sussman and Leverett Chapin) had access to adult asthmatic patients in the Medical Allergy Clinic and to children in the Pediatric Allergy Clinic through the cooperation and courtesy of Drs. Clarence A. Thomas and Alvin E. Keller, and Dr. James C. Overall, respectively. The clinical records contained sensitivity testing data, some of which were quite old. Twenty-two of the 35 children were tested for sensitivity to grasses and trees in 1959 by Study personnel.

Aerometric data were provided by the engineers, based on samples collected and processed according to the methods previously described (21). Annual sulfation values were obtained by calculating the geometric annual mean sulfur trioxide (SO_3) by the lead peroxide candle method. Values were expressed in milligrams of SO_3 per 100 $cm.^2$ per day. For the daily SO_2 data, 24-hour samples of air were passed through a bubbler apparatus and results were expressed in parts per million sulfur dioxide (SO_2) (1 mg. SO_3 per 100 $cm.^2$ per day = approximately 0.035 p.p.m. SO_2).

Meteorologic data were also provided by the engineering program, as described in the preliminary paper on these studies (21). They included wind direction and velocity. Other meteorologic information concerning temperature, humidity, barometric pressure, and precipitation was obtained from the local weather bureau records at Berry Field, Nashville.

From September to November, 1958, and from March to June, 1959, samples of pollens were collected at 10 stations by means of a scotch-tape flag attached to a large pin that could rotate freely in the wind. The flags were collected daily, spread on glass slides and sent to Dr. John Sheldon, University of Michigan, for pollen counts. The data are incomplete, however, because of damage to a number of samples in transit.

TABLE 3

ASTHMATIC ATTACK RATE PER PERSON-DAY BY AGE GROUPS NASHVILLE AIR POLLUTION STUDY

Age Group	Number	Attack Rate
years		
Total	84	0.133
0–14	34	0.105
15–24	6	0.045
25–34	6	0.086
35–44	10	0.140
45–54	15	0.139
55–64	7	0.358
65–74	4	0.120
75–84	2	0.103

RESULTS

Asthmatic attack rates and aerometric factors: The 84 asthmatic patients on the panel reported 3,647 asthmatic attacks during the 27,440 person-days of observation or reports, for an attack rate of 0.133 per patient per day. These were distributed by age as shown in table 3. The range of attack rates was from the low of 0.045 for patients between fifteen and twenty-four years of age, and the high of 0.358 for those fifty-five to sixty-four years. No regular pattern could be shown. In the group under study, those less than thirty-five years of age had lower attack rates than those thirty-five years and older.

The panel patients are grouped in table 4 according to their residence in an area of relatively low, moderate, or high sulfation. Low pollution was considered to be any value below 0.150 mg.; moderate pollution ranged between 0.150 and 0.349 mg.; and high pollution was considered to be represented by values of more than 0.350 mg. These levels are purely arbitrary. They are relative, not absolute. The present state of our knowledge does not permit the establishment of definite maximal allowable concentrations of residential environment air pollutants.

For the whole group of patients the asthmatic attack rate showed a direct correlation with the degree of sulfation. However, the correlation could not be demonstrated for the pediatric asthmatic patients. For the adults the rate was three times as high for those living in the high pollution areas, compared with those in the low. No consistent difference was noted by sex. The breakdown by sex and age produces groups too small to make valid comparisons, but the totals show a higher attack rate for adults than for children.

The asthmatic attack rates for the white and nonwhite patients in the panel are shown in table 5 according to the same three levels of sulfation used in table 4. Here again, a direct correlation

TABLE 4

NUMBER OF REPORTED ASTHMATIC ATTACKS AND ATTACK RATE PER PERSON-DAY FOR ASTHMATIC CHILDREN AND ADULTS BY SEX AND DEGREE OF RESIDENTIAL ENVIRONMENTAL SULFATION*
NASHVILLE AIR POLLUTION STUDY

SO_3 Group	Total				Children				Adults			
	Number of Persons	Number of Reports	Number of Attacks	Attack Rate	Number of Persons	Number of Reports	Number of Attacks	Attack Rate	Number of Persons	Number of Reports	Number of Attacks	Attack Rate
						Total						
Total . . .	84	27,440	3,647	0.133	35	11,579	1,194	0.103	49	15,861	2,453	0.155
I	15	4,745	471	0.099	8	2,408	307	0.127	7	2,337	164	0.070
II	42	13,903	1,541	0.111	18	6,028	471	0.078	24	7,875	1,070	0.136
III	27	8,792	1,635	0.186	9	3,143	416	0.132	18	5,649	1,219	0.216
						Male						
Total . . .	44	14,295	1,882	0.132	25	8,352	562	0.067	19	5,943	1,320	0.222
I	7	2,352	201	0.085	6	1,988	174	0.088	1	364	27	0.074
II	24	7,568	617	0.082	13	4,257	241	0.057	11	3,311	376	0.114
III	13	4,375	1,064	0.243	6	2,107	147	0.070	7	2,268	917	0.404
						Female						
Total . . .	40	13,145	1,765	0.134	10	3,227	632	0.196	30	9,918	1,133	0.114
I	8	2,393	270	0.112	2	420	133	0.317	6	1,973	137	0.069
II	18	6,335	924	0.146	5	1,771	230	0.130	13	4,564	694	0.152
III	14	4,417	571	0.129	3	1,036	269	0.260	11	3,381	302	0.089

* Degree of pollution—based on geometric annual mean sulfation in mg. per 100 $cm.^2$ per day for sampling station nearest the residence of each patient.
I = 0–0.149 mg.
II = 0.150–0.349 mg.
III = 0.350 mg. and over.

between attack rate and pollution level could be demonstrated in the total group, but not uniformly by age and race. Nonwhite patients had a higher attack rate than the white patients. In each group the adults had more attacks per person per day than the children.

Since air pollution levels often vary at different times during the year, an analysis of the attack rates for adults and children in the panel was made by month (table 6). The attack rates for adults were higher in every month except September, 1958, and July, 1959. Contrary to expectations, the highest rates occurred in months when SO_2 was not at its peak. In adults, the highest rate, 0.198, occurred in May, 1959. In children, September, 1958, showed the highest rate of 0.196.

The monthly average values of soiling spots, SO_2, total particulate matter, and wind velocity, with the attack rate for each month from October, 1958, through July, 1959, are shown in table 7. The relationships are shown graphically in figure 5. There was no correlation between the levels of sulfation or the soiling spots and the attack rates. The rates appeared to go down progressively from October, 1958 to March, 1959, while there was a tendency for SO_2 and spot values to rise during this period. The particulate matter tended to follow more closely the course of the attack rates. Wind velocities showed no uniform correlation with the frequency of attacks. During the months when the winds blew the strongest, the attack rates were relatively low. This observation tends to confirm the historical data with regard to sensitivity as shown in table 8. Only 25 per cent of the patients stated that wind was a precipitating factor in attacks, whereas 55 per cent mentioned smoke and 79 per cent incriminated dust as causative factors.

Asthmatic attack rates by occupation were calculated, but the numbers were too small to make meaningful comparisons.

TABLE 5

NUMBER OF REPORTED ASTHMATIC ATTACKS AND ATTACK RATE PER PERSON-DAY FOR ASTHMATIC CHILDREN AND ADULTS BY RACE AND DEGREE OF RESIDENTIAL ENVIRONMENTAL SULFATION*

NASHVILLE AIR POLLUTION STUDY

SO_3 Group	Total				Children				Adults			
	Number of Persons	Number of Reports	Number of Attacks	Attack Rate	Number of Persons	Number of Reports	Number of Attacks	Attack Rate	Number of Persons	Number of Reports	Number of Attacks	Attack Rate
						Total						
Total	84	27,440	3,647	0.133	35	11,579	1,194	0.103	49	15,861	2,453	0.155
I	15	4,745	471	0.099	8	2,408	307	0.127	7	2,337	164	0.070
II	42	13,903	1,541	0.111	18	6,028	471	0.078	24	7,875	1,070	0.136
III	27	8,792	1,635	0.186	9	3,143	416	0.132	18	5,649	1,219	0.216
						White						
Total	43	14,238	1,664	0.117	21	6,987	658	0.094	22	7,251	1,006	0.139
I	14	4,381	466	0.106	8	2,408	307	0.127	6	1,973	159	0.081
II	26	8,849	887	0.100	12	4,243	334	0.079	14	4,606	553	0.120
III	3	1,008	311	0.309	1	336	17	0.051	2	672	294	0.438
						Nonwhite						
Total	41	13,202	1,983	0.150	14	4,592	536	0.117	27	8,610	1,447	0.168
I	1	364	5	0.014	0	0	0	—	1	364	5	0.014
II	16	5,054	654	0.129	6	1,785	137	0.077	10	3,269	517	0.158
III	24	7,784	1,324	0.170	8	2,807	399	0.142	16	4,977	925	0.186

* Degree of pollution—based on geometric annual mean sulfation in mg. per 100 cm.^{2} per day for sampling station nearest the residence of each patient.

I = 0-0.149 mg.
II = 0.150-0.349 mg.
III = 0.350 mg. and over.

TABLE 6

NUMBER OF REPORTED ASTHMATIC ATTACKS BY MONTH AND ATTACK RATE PER PERSON-DAY FOR CHILDREN AND ADULTS

NASHVILLE AIR POLLUTION STUDY

Month and Year	Total				Children				Adults			
	Number of Person-Days of Report	Number of Attacks	Attack Rate: Attacks per Person per Day	Per Cent of Total Number of Attacks	Number of Person-Days of Report	Number of Attacks	Attack Rate: Attacks per Person per Day	Per Cent of Total Number of Attacks	Number of Person-Days of Report	Number of Attacks	Attack Rate: Attacks per Person per Day	Per Cent of Total Number of Attacks
Total	27,440	3,647	0.133	100.0	11,579	1,194	0.103	100.0	15,861	2,453	0.155	100.0
1958												
July 14-31	1,147	138	0.120	3.8	404	45	0.111	3.8	743	93	0.125	3.8
August	2,305	384	0.167	10.5	878	134	0.153	11.2	1,427	250	0.175	10.2
September	2,393	432	0.181	11.8	969	190	0.196	15.9	1,424	242	0.170	9.9
October	2,471	439	0.178	12.0	1,028	164	0.160	13.7	1,443	275	0.190	11.2
November	2,339	362	0.155	9.9	999	130	0.130	10.9	1,340	232	0.173	9.4
December	2,381	334	0.140	9.2	1,020	118	0.115	9.9	1,361	216	0.159	8.8
1959												
January	2,378	243	0.102	6.7	1,019	58	0.057	4.9	1,359	185	0.136	7.5
February	2,143	206	0.096	5.6	924	46	0.050	3.8	1,219	160	0.131	6.5
March	2,328	201	0.086	5.5	1,016	31	0.031	2.6	1,312	170	0.130	6.9
April	2,240	255	0.114	7.0	972	74	0.076	6.2	1,268	181	0.143	7.4
May	2,277	348	0.153	9.5	1,006	96	0.095	8.0	1,271	252	0.198	10.3
June	2,198	234	0.106	6.4	970	72	0.074	6.0	1,228	162	0.132	6.6
July 1-12	840	71	0.084	1.9	374	36	0.096	3.0	466	35	0.075	1.4

TABLE 7

ASTHMATIC ATTACK RATE PER PERSON-DAY AND MONTHLY AVERAGE VALUE OF DAILY AIR POLLUTION AND WIND VELOCITY

NASHVILLE AIR POLLUTION STUDY

Month and Year	Attack Rate: Attacks per Person per Day	Spots COHS*	Sulfur Dioxide	Total Particulate	Wind
		per 1,000 ft.	*parts per million*	*γ/meter³*	*miles/day*
October, 1958...	0.178	1.119	0.010	195.73	59.13
November.....	0.155	1.455	0.025	178.14	92.75
December.....	0.140	2.340	0.042	177.21	89.71
January, 1959..	0.102	2.415	0.032	157.40	121.61
February.....	0.096	2.312	0.025	153.03	115.34
March.....	0.086	1.725	0.018	130.82	135.14
April.....	0.114	0.863	0.007	155.14	116.88
May.....	0.153	0.375	0.001	123.17	76.99
June.....	0.106	0.291	0.001	125.64	68.93
July.....	0.084	0.331	0.000	110.96	58.86

* COHS—Concentration of haze and smoke

An analysis was made of attack rates for each day of the week, and they were compared with the air pollution levels on these days for the purpose of determining what influence industry might have had on all of the variables. With most industries operating on a five-day week, variations in the levels of pollutants emitted by industry should have been noticeable. The data are shown in tables 9 and 10 and are graphically presented in figure 6. For children the highest attack rates occurred on Sunday. The second highest rates occurred on Monday, after which it declined, only to rise gradually through the week, with a sudden sharp rise on Saturday. The pattern for adults was somewhat different. For them the highest rates were noted on Saturday, the low on Monday, followed by an almost steady rise through the week. None of the aerometric variables followed this pattern. Sulfur dioxide was at its lowest during the week when industry was in full swing, and highest over the week-end

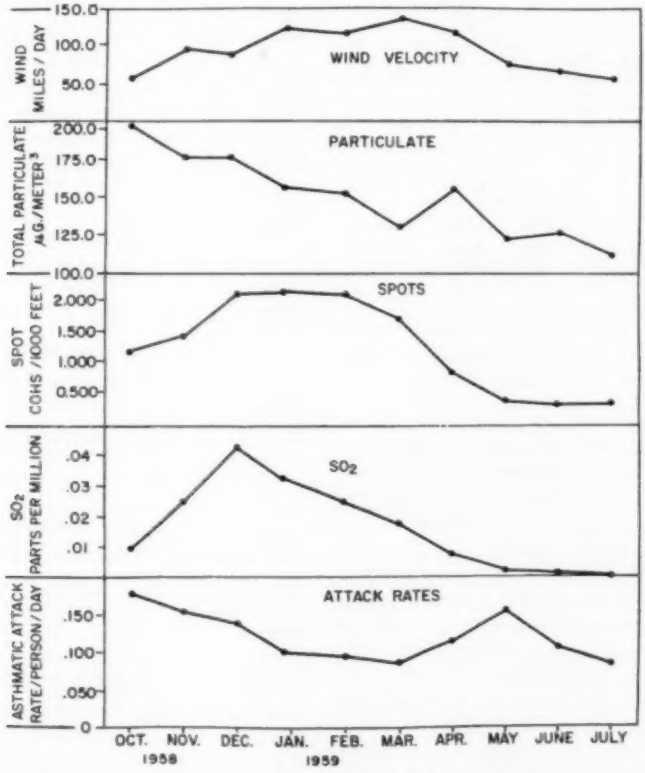

FIG. 5. Monthly average of asthmatic attack rates, air pollution levels, and wind velocity, October, 1958–July, 1959.

TABLE 8

NUMBER AND PERCENTAGE OF PEDIATRIC AND ADULT ASTHMATIC PATIENTS WITH HISTORIES OF SENSITIVITY TO METEOROLOGIC, ATMOSPHERIC, AND OTHER FACTORS
NASHVILLE AIR POLLUTION STUDY

	Total		Children		Adults	
	Number	Per Cent	Number	Per Cent	Number	Per Cent
Climatologic conditions:						
Changes in temperature	51	61	21	60	30	61
Damp	55	65	23	66	32	65
Wind	21	25	8	23	13	27
Atmospheric conditions:						
Smoke	46	55	10	29	36	73
Dust	66	79	19	54	47	96
Fumes	50	60	12	34	38	78
Heating	16	19	3	9	13	27
Drafts	32	38	8	23	24	49
Air conditioning	13	15	1	3	12	24
Other conditions:						
Sensitivity to foods	19	23	4	11	15	31
Emotions	53	63	19	54	34	69

TABLE 9

ASTHMATIC ATTACK RATE PER PERSON-DAY BY DAY OF WEEK FOR CHILDREN AND ADULTS
NASHVILLE AIR POLLUTION STUDY

Day	Total	Children	Adults
Total	0.133	0.103	0.155
Monday	0.126	0.109	0.139
Tuesday	0.122	0.097	0.141
Wednesday	0.132	0.097	0.157
Thursday	0.131	0.099	0.154
Friday	0.137	0.100	0.164
Saturday	0.149	0.108	0.171
Sunday	0.137	0.112	0.156

when plants were idle. This appears to indicate that the SO_2 had more of a domestic than an industrial source. Soiling followed a similar pattern to SO_2, a not unexpected finding, since both may be traced to coal usage. Particulate matter was at its highest on Tuesday and lowest on Sunday, compatible with an industrial source.

The occurrence of asthmatic attacks during the night or early morning is well known to clinicians and is stated in textbooks. In the present study it was possible to tabulate attacks by time of occurrence because the report cards carried such information. The day was divided into six periods of four hours, beginning at midnight. The tabulations are shown in table 11. The largest proportion of first attacks during a day occurred between midnight and 3:59 a.m. Adult asthmatic patients reported 33.5 per cent of their first attacks, while even more children (44.3 per cent) experienced their first attacks during this period. Between midnight and 8:00 a.m., 57.2 per cent of adults and 61 per cent of the children had their first attacks.

The sulfur dioxide data for Nashville were col-

TABLE 10

ASTHMATIC ATTACK RATES PER PERSON-DAY AND MEAN DAILY AVERAGE OF AIR POLLUTION AND WIND VELOCITY
NASHVILLE AIR POLLUTION STUDY

Day	Attack Rate per Person-Day	Spots COHS*	Sulfur Dioxide	Total Particulate	Wind
		per 1,000 ft.	*parts per million*	*γ/meters³*	*miles/day*
Total	0.133	1.317	0.016	150.68	93.33
Monday	0.126	1.439	0.019	149.73	86.42
Tuesday	0.122	1.343	0.016	169.17	93.03
Wednesday	0.132	1.202	0.015	151.65	96.64
Thursday	0.131	1.300	0.015	151.18	96.31
Friday	0.137	1.209	0.014	153.63	96.08
Saturday	0.149	1.393	0.016	151.76	91.16
Sunday	0.137	1.341	0.017	127.53	93.43

* COHS—Concentration of haze and smoke

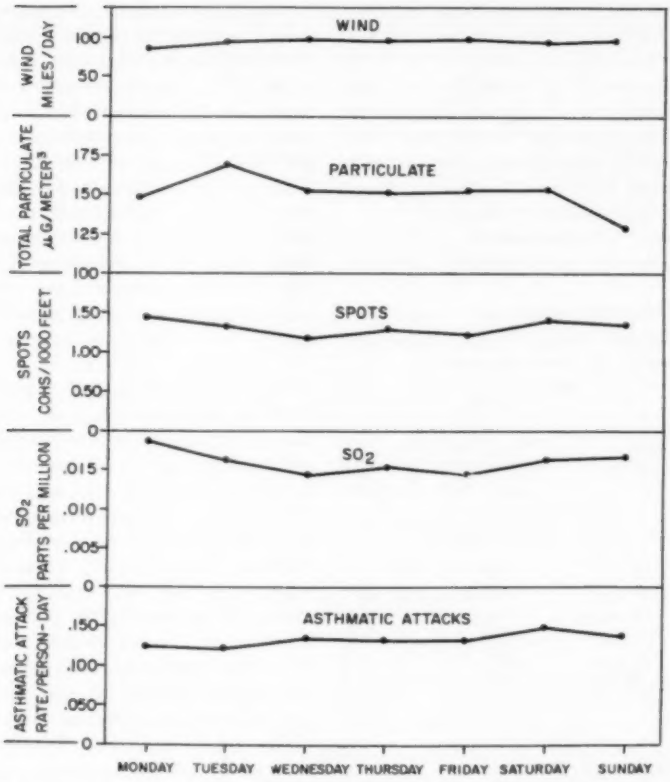

FIG. 6. Asthmatic attack rates, air pollution levels, and wind velocity, by day of the week, October, 1958–July, 1959.

lected over a period of 285 days. The median sulfur dioxide was determined to be 0.01 parts per million (p.p.m.). For the days with SO_2 above the median the asthmatic attack rate was 0.131 per person-day, with an average of 9.4 daily attacks reported. The attack rate on the days below the median was 0.127, and the number of average daily attacks was 7.5. The differences were not significant. However, when attack rates on the thirty days with the highest SO_2 levels were compared with the rates on the forty-six days with the lowest levels, the difference in the respective rates of 0.130 and 0.110 was significant at the 5 per cent level. The daily data on asthmatic attacks were deferred by one day to take account of possible delayed reactions to SO_2. By this procedure the attack rates for the highest and lowest value days were different at the 1 per cent level. In a community where SO_2 values usually are not extraordinarily high at their worst, it may not be possible to make valid comparisons except at the extremes.

Meteorologic factors: The influence of meteorologic factors on the occurrence of asthmatic attacks is disputed. Peterson (22) contends that an atmospheric change is the dominant force to which physiologic adjustment must be made by the individual, and that abrupt alterations often initiate clinical symptoms. He was not referring to bronchial asthma specifically. Markow and Reicher (23) state that "daily changes in meteorologic factors such as temperature, relative humidity, barometric pressure and rainfall have been shown to be related to outbreaks of asthma." They note, however, that these observations covered only brief periods of time. In their two-year study of a group of asthmatic patients they noted that a graph of the asthma symptoms was similar

for both years and showed a bimodal distribution, peaks occurring in May and January. Patients in the Nashville study showed peaks in May and September. Markow and Reicher found some association between asthmatic attacks and temperature, humidity, and rainfall, but these were not clear-cut. They did not find any relationship between the concentration of atmospheric pollens and asthmatic symptoms.

The association of temperature, humidity, barometric pressure, and wind velocity on asthmatic attacks was analyzed by computing Pearson's product moment correlation coefficients between the meteorologic parameters and the asthmatic attacks. The results were so variable that no trend could be shown (table 12). When comparisons were made of attack rates on the days with the highest and lowest values for each meteorologic parameter, the only significant differences found were for wind velocity. Attack rates were much higher on days with the lowest wind movement compared with days with high velocity. This observation supports the expectation that, if air pollutants are related to asthmatic attacks, days on which high winds disperse the pollutants should show low attack rates.

TABLE 11

TIME OF THE FIRST ASTHMA ATTACK EACH DAY
NASHVILLE AIR POLLUTION STUDY

Time Period	Adults		Children	
	Number of Attacks	Per cent of Total	Number of Attacks	Per cent of Total
Total	2,093	100.0	909	100.0
12:00 to 3:59 a.m.	702	33.5	403	44.3
4:00 a.m. to 7:59 a.m.	497	23.7	152	16.7
8:00 a.m. to 11:59 a.m.	161	7.7	37	4.1
12:00 p.m. to 3:59 p.m.	121	5.8	73	8.0
4:00 p.m. to 7:59 p.m.	324	15.5	123	13.5
8:00 p.m. to 11:59 p.m.	288	13.8	121	13.3

TABLE 12

LINEAR CORRELATION COEFFICIENTS BETWEEN VARIOUS MEASURES OF METEOROLOGIC VARIABLES AND DAILY ASTHMATIC ATTACK RATES
NASHVILLE AIR POLLUTION STUDY

Month	Wind	Temperature	Barometric Pressure	Relative Humidity
1958				
July	*	−0.210	0.271	0.148
August	*	0.012	0.054	0.018
September	*	−0.423†	−0.549‡	0.389†
October	0.012	0.288	0.398†	−0.087
November	−0.180	0.231	−0.265	0.153
December	0.074	−0.226	−0.078	−0.317
1959				
January	−0.075	−0.254	0.517‡	−0.457‡
February	−0.103	0.170	0.205	−0.215
March	−0.023	0.161	−0.225	0.178
April	−0.350†	−0.453†	0.338	0.150
May	0.052	−0.400†	−0.015	−0.269
June	−0.021	−0.690‡	−0.195	−0.439†
July	0.070	0.046	−0.430†	−0.111

* Data not available
† Significant at 5%
‡ Significant at 1%

Pollen factor: The effect of pollen in the ambient air on the frequency of asthmatic attacks was investigated as another possible asthma-precipitating factor that needed to be controlled if the effect of other air pollutants was to be evaluated properly. The pollen counts were compared to asthmatic attack rates by computing Pearson's coefficients. The only value that was statistically significant was that for oak pollen at Station 60 for April, 1959. The correlation coefficient of 0.673 is significant at the 1 per cent level. However, because of the small number of observations, caution must be used in attaching any real meaning to the analysis.

Pulmonary function: Pulmonary function tests, as described above, were done on 34 adults and 22 children in the asthma panel. The data have not been analyzed fully at this time, so a final evaluation will have to be made in a subsequent report.

Considerable variation in pulmonary function was noted between individuals, and from time to time in the same individual. Whether these variations resulted from actual changes in function, or were the result of different testing techniques or variability in the cooperation of the patient, cannot be said at this time. Probably all of the factors operated to some degree. Certainly, in a test in which the cooperation of the patient is of major importance, a subject may have to learn how to perform at his maximal capacity as tests are repeated. Among some of the adults, and in many of the children, the one-second timed vital capacity improved after several tests; but it declined in others.

Stuart-Harris and Hanley (24) report that the one-second timed vital capacity per cent of vital capacity was 80 in normal subjects and

only 40 in asthmatic patients. In the Study panel, most of the patients showed values well over 40.

A comparison of asthmatic attack rates was made for the 8 patients with one-second timed vital capacity per cent of vital capacity of less than 50, and for 8 patients with values of more than 75 per cent. In the low test group, the attack rate of 0.226 was seven times as high as the rate of 0.033 in the high group. It appears then that the pulmonary function is impaired in asthmatic patients. Whether the function varies with the concentration of air pollutants from day to day remains to be determined.

Discussion

Many factors have been listed as the cause of bronchial asthma. A great variety of allergens has been implicated. Meteorologic and aerometric factors have been mentioned in precipitating attacks. The emotional factor is considered by many to be of major importance. In children especially, the influence of other conditions such as acute respiratory infections is thought to be considerable. With so many variables the problem of establishing adequate controls in a study becomes all but insoluble. It may be possible to keep some factors constant while others are varied, one at a time, but control of such imponderables as emotional factors would offer considerable difficulty. Clear-cut cause and effect relationships would be exceptional.

The results thus far established in the Study appear to show a direct correlation between asthmatic attack rates and sulfation levels for the whole group of asthmatics. However, the correlation is established by the experience of the adults, who influence the total rates unduly because of their preponderance in the group. The pediatric asthmatics show no such direct correlation. The disease, bronchial asthma, is considered by many clinicians to be distinctly different in children and adults. In the former there is a great tendency to the precipitation of attacks by other conditions such as upper respiratory infections. Whether such differences can account for the strong correlation of attacks and sulfation in the adult group and the lack of correlation in the children cannot be stated, but it may not be reasonable to compare the two groups or to treat them similarly.

The Study reveals that asthmatic patients living in areas of relatively high sulfation had a higher attack rate than those living in an area of relatively low sulfation. It might be expected, therefore, that for the study group as a whole the months in which SO_2 is highest would also show the highest asthmatic attack rate. This could not be shown. However, analyses of monthly attack rates by degree of pollution (which have not yet been completed) may tend to show the same correlation observed on an annual basis.

Study of the extremes in SO_2 variations promises to be rewarding. Additional effort will be devoted to analyses of other atmospheric and meteorologic variables to determine their possible relation to variations in asthmatic attack rates.

One of the great problems in studies on asthmatic patients is to define an "asthmatic attack." Some patients have some degree of wheezing almost constantly. In them it would be difficult to define the onset or end of an attack. There is considerable individual variation in defining an attack. In a program of reporting by patients, the errors inherent in subjective evaluations cannot be avoided. Precise and objective evaluations would greatly improve the quality of the data. If a reliable pulmonary function test could be developed that required a minimum of effort and cooperation on the part of the patient, more precise information could be obtained by comparing variations in pulmonary function with changes in the concentration of atmospheric pollutants.

Summary

A group of 84 patients with bronchial asthma, composed of 49 adults and 35 children, reported 3,647 asthmatic attacks during 27,440 person-days of observation or an over-all attack rate of 0.133 per person-day. In adults the asthmatic attack rate varied directly with the level of sulfation in their residential environment.

Attack rates on days with the highest and lowest sulfur dioxide values were significantly different. When the attack rates were shifted over one day to take account of possible delayed effects, the differences were even more significant. The influence of temperature, humidity, and barometric pressure on the asthmatic attack rate could not be demonstrated, but wind velocity showed an inverse relationship. Pulmonary function tests indicated that subjects with a one-second timed vital capacity of less than 50 per cent of the vital capacity had significantly higher attack rates than patients with more than 75 per cent function.

Sumario

Estudio de la Contaminación del Aire de Nashville: I. El Bióxido de Azufre y el Asma Bronquial. Comunicación Preliminar

Un grupo de 84 enfermos de asma bronquial, compuesto de 49 adultos y 35 niños, comunicó 3,647 accesos asmáticos durante 27,440 días-personas de observación, o sea una tasa global de accesos de 0.133 por día-persona. En los adultos, la tasa de accesos asmáticos varió directamente de acuerdo con el tenor de azufración en el ambiente residencial.

Las tasas de accesos en los días de cifras máximas y mínimas de bióxido de azufre fueron notablemente distintas. Cuando se interpuso un día, las diferencias fueron todavía más notables. No pudo descubrirse el influjo de la temperatura, la humedad y la presión barométrica sobre la tasa de accesos asmáticos, pero la velocidad del viento mostró una relación inversa. Las pruebas de la función pulmonar indicaron que los sujetos con una capacidad vital calculada al segundo de menos de 50 por ciento tenían tasas significativamente más altas de accesos que los que tenían una función de más de 75 por ciento.

Resume

Etude sur la population de l'air de Nashville: I. Dioxyde de soufre et asthme. Un rapport préliminaire

Un groupe de 84 malades, ayant de l'asthme bronchique composé de 49 adultes et 35 enfants, fit le rapport de 3.647 crises d'asthme pendant 27.440 jours d'observation par personne soit un taux général de crise de 0.133 par jour par personne. Chez les adultes, le taux de crises d'asthme varie directement avec la quantité de soufre du milieu où ils habitent.

Le nombre de crise les jours où il y en eu le plus et les jours ou il y eut le moins de dioxyde de soufre étaient nettement différentes. Quand on arriva à un jour, les différences furent encore plus importantes. L'influence de la température, de l'humidité, et de la pression barométrique sur la crise d'asthme ne put pas être prouvée, mais la vitesse du vent se montra être en rapport inverse. Les tests de la fonction pulmonaire indiquèrent que les sujets ayant une capacité vitale, calculée à 1 seconde, de moins de 50% de la capacité vitale, avaient un nombre de crises nettement plus élevé que les malades ayant plus de 75% de leur fonction.

Acknowledgment

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CHEMOTHERAPEUTIC STUDIES ON A NEW ANTIFUNGAL AGENT, X-5079C, EFFECTIVE AGAINST SYSTEMIC MYCOSES[1, 2]

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Introduction

Among the modern chemotherapeutic agents employed for the treatment of deep mycotic infections have been the arsenicals, the antimony compounds, ethyl vanillate, stilbamidine, the sulfonamides, and a number of drugs of microbial origin (1–3). The most useful have been the drugs of microbial origin, penicillin still being the treatment of choice in actinomycosis, and amphotericin B covering a rather broad spectrum but limited in usefulness because of toxic side effects. Since the more potent chemotherapeutic agents for the control of experimental systemic mycoses have been found among materials of microbial origin, samples of broths that had contained microbial growth have been investigated for antifungal properties in this laboratory. The present report describes the laboratory studies of a substance designated antifungal agent X-5079C, the first such agent of a nonpolyene nature which has shown a high degree of activity against certain systemic mycotic infections.

Isolation and Properties

The drug X-5079C is produced by Streptomyces nov. sp. X-5079 when grown in submerged culture on a soy-bean flour–sugar–salts medium. The antimicrobial agent can be readily differentiated from known substances by a combination of the following properties: (*1*) its polypeptide nature and sulfur content of 12 to 14 per cent, (*2*) solubility of the sodium salt in water, (*3*) a narrow *in vitro* antimicrobial spectrum, and (*4*) systemic *in vivo* activity against *Histoplasma capsulatum* coupled with low toxicity.

The steps in isolation of the material can be followed by its activity *in vitro* against *Paecilomyces varioti* using a cup-plate agar-diffusion method. Assay plates containing a 20-ml. base layer of agar are covered with 4 ml. of molten agar seeded with a heavy spore suspension of the test organism, and are incubated at 35°C. for eighteen hours. At the end of the incubation period the diameter of the inhibition zones is measured, and one unit of activity has been arbitrarily assigned to that amount of material which, when dissolved in water, produces a 20-mm. inhibition zone. *In vitro* assay results roughly parallel the *in vivo* activity found against *H. capsulatum*.

The purified substance assays approximately 220 to 250 *P. varioti* units per mg. It is a white, amorphous powder of polypeptide nature. Five of the amino acids have been tentatively identified, after acid hydrolysis of the agent, as cysteic acid, aspartic acid, glycine, threonine, and proline. The purified substance is inactive *in vitro* on assay plates against 18 nonpathogenic yeasts and 21 saprophytic bacteria commonly used for assay procedures. Strains of Penicillium, Aspergillus, Alternaria, Fusarium, and Rhizopus are susceptible *in vitro* to the drug but give only hazy inhibition zones on the assay plates.

By the broth-dilution method, X-5079C shows no effect at concentrations of 1,000 to 2,500γ per ml. against: (*1*) pathogenic bacteria: *Streptococcus hemolyticus, Diplococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Salmonella typhosa*, and *Mycobacterium tuberculosis*; (*2*) the dermatophytes: *Trichophyton mentagrophytes, Trichophyton purpureum, Microsporum audouini*, and *Microsporum lanosum*; and (*3*) *Candida albicans*. By the same method, *in vitro* activity has been observed against *H. capsulatum* and *Blastomyces dermatitidis* at concentrations of 1,000γ per ml. or more.

A purified batch of X-5079C used for clinical trial, and designated /29, when tested for acute toxicity in white mice gave LD_{50}'s of >2,000 mg. per kg. subcutaneously, >2,000 mg. per kg.

[1] From the Department of Chemotherapy, Hoffmann-La Roche Inc., Nutley, New Jersey.

[2] Presented before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 22, 1961.

intraperitoneally, and >1,000 mg. per kg. intravenously. Sprague-Dawley rats weighing approximateiy 200 to 250 gm. received 100 mg. per kg. of the agent intramuscularly daily for one, three, six, and eight days. No grossly visible toxic manifestations or local tissue damage were observed. After three daily injections, serum lactic dehydrogenase activity was somewhat above normal. Histologically, only slight to moderate hydropic changes of the hepatic cytoplasm were observed. The histopathologic findings at the end of the maximal treatment period were of such degree that they were judged to be nonpathologic variations from normal.

Two monkeys received 50 mg. per kg. and one monkey received 100 mg. per kg. of purified X-5079C subcutaneously for a total of thirty-four consecutive days. No gross toxic symptoms were observed. Using the assay procedure with *P. varioti* for the detection of blood and urine concentrations, it has been found that, twenty-four hours after the last administration of the drug to the monkeys, 10 per cent of the agent was recoverable from erythrocytes after lysis and 20 per cent could be recovered in a twenty-four-hour urine sample. The standard assay procedure also showed the presence of the substance in the serum, blood, and urine of rats and rabbits.

The drug X-5079C has been found to be active against the experimental infection of mice with *H. capsulatum* and *B. dermatitidis*.

The experimental method employed is as follows: The organism is grown on egg-potato slants for seven to eight days in order to obtain growth in the yeastlike phase (4). The growth is then washed off from the surface of the medium in 5-ml. saline, and the suspension is spun in the centrifuge at 100 r.p.m. for five minutes. Centrifugation is employed so that clumps of cells will settle out at the bottom of the tube while single cells remain in the supernatant. The supernatant is decanted and the number of cells per milliliter is determined by hemocytometric count using the technique employed for counting erythrocytes. The suspension is then diluted to contain 5 million yeastlike cells per ml. of *H. capsulatum* or 1 million yeastlike cells per ml. of *B. dermatitidis*.

To accomplish the infection of mice, 0.1 ml. of a 1 per cent suspension of cortisone in saline is injected intramuscularly in the morning and afternoon of the day before infection. On the day of infection the animals receive 0.1 ml. of the 1 per cent suspension of cortisone in the morning, followed by 0.5 ml. of the suspension of 5 million cells per ml. of *H. capsulatum* or 1 million cells per ml. of *B. dermatitidis* intravenously. On the same afternoon, a fourth treatment of cortisone is administered. Fifth and sixth injections of cortisone may or may not be employed on the morning or afternoon of the day following infection.

Animals infected in the above manner usually succumbed between the seventh and fourteenth days after infection. In the case of *H. capsulatum*, no gross pathologic lesions were observed. However, contact smears of the spleen stained by Giemsa's method showed the presence of numerous organisms of *H. capsulatum*, an observation which could also be confirmed by culture.

Animals infected with *B. dermatitidis* showed the presence of gross lesions in the lungs from which the fungus could be cultured.

For chemotherapeutic studies, treatment with the drug is started on the day of infection and continued until all animals have succumbed, or for a maximum of twenty-eight days. The following criteria are used for the evaluation of antifungal effectiveness: (*1*) In Histoplasma infections, contact smears are made from the spleens, and only the complete absence of microorganisms on microscopic examination is considered to indicate efficacy. (*2*) In Blastomyces infections, absence of gross lesions in the lungs and cultures negative for the organisms are the criteria.

Using the above-described experimental procedures, the antifungal agent X-5079C gave a CD_{50} of 2 to 5 mg. per kg. subcutaneously for *H. capsulatum*, and 15 to 25 mg. per kg. subcutaneously for *B. dermatitidis*. Orally, the agent was 40 to 100 times less active against *H. capsulatum*, the CD_{50} being 200 mg. per kg. *per os*.

Using essentially the same method, the drug was tested against the systemic *C. albicans* infection of white mice. In this case the infective dose was 50,000 organisms, and the criteria for evaluation were the absence of renal lesions and renal cultures negative for *C. albicans*. Under these conditions the drug was inactive at doses of >200 mg. per kg. subcutaneously.

The drug has also been tested at the maximal tolerated dose and found to be inactive *in vivo* against the following infections in mice: (*1*) bacteria: *Str. hemolyticus*, *D. pneumoniae*, *Staph. aureus*, *S. schottmuelleri*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *M. tuberculosis*, and *M. leprae murium*; (*2*) protozoa: *Trichomonas vaginalis*, *Trypanosoma equiperdum*, and *Endamoeba histolytica*; (*3*) viruses:

murine poliomyelitis and influenza A; and (*4*) tumors: sarcoma 180 and Ehrlich ascites.

Summary

A new polypeptide antimicrobial drug, X-5079C, is described which has marked activity against *Histoplasma capsulatum* and *Blastomyces dermatitidis* infections in mice. The substance is unusual in that it exerts only slight activity against *H. capsulatum* and *B. dermatitidis in vitro*.

Its antimicrobial spectrum is limited *in vitro* and/or *in vivo* in that it is inactive against nonpathogenic fungi and bacteria and pathogenic bacteria, protozoa, viruses, tumors, and dermatophytes. The antifungal agent is of low toxicity in mice, rats, and monkeys. A method for assaying blood, serum, and urine concentrations using *Paecilomyces varioti* as a test organism is outlined.

Sumario

Estudios Quimioterapéuticos de un Nuevo Agente Antifungoso, X-5079C, Eficaz contra las Micosis Generalizadas

Se describe un nuevo agente antimicrobiano polipéptido, X-5079C, que posee notable actividad contra las infecciones por *Histoplasma capsulatum* y *Blastomyces dermatitidis* en los ratones. La sustancia es excepcional en que no despliega más que ligera actividad contra el *H. capsulatum* y el *B. dermatitidis in vitro*.

El espectro antimicrobiano queda limitado *in vitro* y/o *in vivo* por ser inactivo contra los hongos y las bacterias anapatógenos y contra bacterias, protozoos, virus, tumores y dermatofitos que son patógenos. El nuevo agente antifungoso muestra poca toxicidad en ratones, ratas y monos. Se bosqueja un método para valorar las concentraciones en la sangre, el suero y la orina, usando el *Paecilomyces varioti* como microbio de ensayo.

Resume

Etudes chimiothérapeutiques d'un nouvel agent antifungique, X-5079C, actif dans les mycoses systémiques

Les auteurs décrivent un nouvel agent, un polypeptide, antimicrobien, X-5079C, qui a une activité marquée sur les infections à *Histoplasma capsulatum* et *Blastomyces dermatitidis* chez les souris. Cette substance est originale par le fait qu'elle n'exerce qu'une légère action sur *H. capsulatum* et *B. dermatitidis in vitro*.

Son spectre antimicrobien est limité *in vitro* et/ou *in vivo* n'a pas d'action sur les fungi et les bactéries non pathogènes et les bactéries pathogènes, protozoaires, virus, tumeurs et dermatophytes. Le nouvel agent antifungique a une toxicité faible chez les souris, les rats, et les singes. Les auteurs décrivent une méthode d'étude des concentrations dans le sang, le sérum et l'urine, utilisant *Paecilomyces varioti* comme organisme de réaction.

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CHEMOTHERAPEUTIC AND TOXIC ACTIVITY OF THE ANTIFUNGAL AGENT X-5079C IN EXPERIMENTAL MYCOSES[1, 2]

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(Received for publication May 25, 1961)

Introduction

In the continuing search for more effective and less toxic antifungal agents for clinical use in the treatment of mycotic infections, therapeutic trial in experimentally infected animals plays an essential role. *In vitro* screening of drugs selects many which are either too toxic for clinical use, are not absorbed, or are inactivated in the body. This is not the only disadvantage of *in vitro* screening. An occasional drug with potentialities for successful clinical use produces such a narrow zone of inhibition on the test plate that it would be discarded routinely without further study. An antifungal agent which falls into the latter category is the subject of this report.

Materials and Methods

Dr. E. Grunberg of Hoffmann-La Roche, Inc., submitted to the Mycology Section, National Institute of Allergy and Infectious Diseases, in September, 1956, an antifungal agent now identified by the code number X-5079C (1). In his *in vivo* screening program he had found this drug effective against experimental histoplasmosis, and he requested that it be retested by the methods used in this laboratory. The agent was difficult to prepare and, as it was available in only very small quantity, it was supplied to this laboratory for exclusive trial.

The drug was tested *in vitro* in broth-dilution cultures and in assay discs placed on seeded agar plates, although Dr. Grunberg had informed us that it was relatively inactive *in vitro*. Dr. J. Berger generously supplied details of the *in vitro* test methods he had used in the laboratories at Hoffmann-La Roche, and gave us a culture of *Paecilomyces varioti* which he had determined by comparative studies to be susceptible to the drug (1).

The toxicity of X-5079C (Lot No. 16) for mice was tested by intraperitoneal and intravenous injections of the agent in increasing doses in both acute and chronic toxicity tests. The tissues of the mice were fixed in 10 per cent formalin or in buffered formalin sublimate (B5), embedded in paraffin, and stained routinely with azure-eosin. Frozen sections of liver and kidney were regularly stained with Oil Red O for lipid. When indicated, sections were stained for hemosiderin by Perls' method, for collagen by the Van Gieson method, and for Histoplasma by methanamine silver. The liver, kidney, spleen, adrenal gland, pancreas, diaphragm, small intestines, mesentery, and abdominal lymph nodes were examined routinely. The heart and lungs of most mice and the thymus, uterus, urinary bladder, stomach, and large intestine of a few mice were included in the histologic examination.

The antifungal agent was tested by the standardized experimental procedure long used in the Mycology Section and reported in various papers. The procedure may be outlined as follows: By prior trials, an infecting dose of the test fungus is selected which kills all untreated mice during the third postinfection week. The dose selected depends upon the species of fungus and the virulence of the strain. When, as often happens, an unintentionally severe infection kills the control mice during the second postinfection week, the experiment still yields valid data, although it submits the drugs under trial in that experiment to an unusually severe test. Swiss, white, female mice[3] weighing 17 to 20 gm., with a maximal weight-spread of 2 gm. in any one experiment, are used routinely in this laboratory. Mice are infected by injecting into a lateral tail vein (2) 0.5 ml. of a suspension of cells of the test fungus. Each experiment must contain at least one untreated group of 10 mice. Additional control groups of 10 mice each should include a group receiving injections of the drug's solvent or suspending fluid only. If the toxicity of the drug has not been tested adequately before, a group of uninfected mice injected with the drug on the schedule used in experimental therapy should also be included. An experiment may contain as many additional groups of 10 mice each as the time schedule permits, and these groups should be used in tests of various doses of the drug or of several other drugs tested simultaneously in the same experiment.

The treatment of the mice is begun by intraperitoneal injection of a suspension or solution of the drugs under test twenty-four to seventy-two

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 22, 1961.

[3] National Institutes of Health general-purpose mice.

hours after experimental infection. In investigations of the potentialities of a drug, the interval between infection and start of treatment has been varied from twenty-four hours to seven days, the duration of treatment from two days to thirty-four days, and the dosage varied within tolerated limits (tables 1–3). The dosage schedule has been varied so as to give three, five, or seven treatments per week, and the routes of administration have included the intraperitoneal, intravenous, subcutaneous, and oral methods, the latter *ad libitum* in food or by canula. The present routine procedure is to infect one group of mice with *Histoplasma capsulatum*, and a second group with *Cryptococcus neoformans* on a Friday, institute treatment on the following Monday, and administer the drug daily or five times per week for twenty days to all mice surviving for that period. If the drug under test is known to produce persistent tissue or serum concentrations, treatments may be reduced to three per week. If the drug is active against the two standard test fungi, it is then tested against *Blastomyces dermatitidis*, *Coccidioides immitis*, *Candida albicans*, and other pathogens.

TABLE 1

RESPONSE OF MICE WITH EXPERIMENTAL HISTOPLASMOSIS TO TREATMENT WITH X-5079C, LOT NO. 16

Experiment Number	Number of Mice	Drug Dose in Mg. per Kg.	Delay before Therapy: Days	Duration of Therapy: Days	Schedule of Injections*	Number of Deaths	Day of Median Death	Day of Last Death	Day Survivors Were Killed	Mice Cultured: Positive/Total	Comments
1	16	0	—	—	—	15	9.5	24	24	—	Mouse 15 died on day 15
	16	125.0	1	24	Daily	1	—	20	38	0/15	4 mice killed on day 25
2	10	0	—	—	—	10	10.5	14	—	—	
	10	5.3	4	7	Daily	0	0	0	25	10/10	
	10	5.3	4	14	Daily	1	—	16	25	9/9	
	10	10.6	4	7	Daily	0	0	0	25	10/10	
	10	10.6	4	14	Daily	0	0	0	25	10/10	
	10	21.2	4	7	Daily	1	—	6	25	9/9	
	10	21.2	4	14	Daily	2	—	25	25	8/8	
	10	118.0	4	2	Daily	3	—	6	25	7/7	
3	10	0	—	—	—	9	11.0	23	64	1/1	
	10	25.0	2	46	5 times weekly	0	—	6	64	0/10	
	10	50.0	2	46	5 times weekly	1	—	8	64	0/9	
	10	100.0	2	46	5 times weekly	2	—	16	64	0/8	
	10	100.0†	6	46	5 times weekly	3	—	9	64	2/7	
4	10	0	—	—	—	10	—	27	—	—	
	10	5.0	1	7	5 times weekly	2	—	124	133	5/8	Only 1 or 2 of 4 cultures from each mouse positive
	10	5.0	1	14	5 times weekly	1	—	84	133	3/9	
	10	5.0	1	14	5 times weekly‡	0	—	—	133	6/10	
	10	5.0	1	14	5 times weekly§	1‖	—	—	133	5/9	
5	10	0	—	—	—	9	17.4	36	48	1/1	
	10	60.0	1	34	5 times weekly	0	—	—	48	0/10	
6	10	0	—	—	—	10	12.0	15	—	—	
	10	240.0	1	29	Daily	0	—	—	38	0/10	

* Injections intraperitoneal unless indicated otherwise.
† Dose 25–50 mg. per kg. during first 4 days of therapy.
‡ Intravenous administration of drug.
§ Subcutaneous administration of drug.
‖ Traumatic death.

TABLE 2

RESPONSE OF MICE WITH EXPERIMENTAL BLASTOMYCOSIS AND COCCIDIOIDOMYCOSIS TO TREATMENT WITH X-5079C, LOT NO. 16

Experiment Number	Number of Mice	Drug Dose in Mg. per Kg.	Delay before Therapy: Days	Duration of Therapy: Days	Schedule of Injections	Number of Deaths	Day of Median Death	Day of Last Death	Day Survivors Were Killed	Mice Cultured: Positive/Total	Comments
Blastomycosis											
1	10	0	—	—	—	10	6.7	8	48	—	
	10	60	1	34	5 times weekly	7	13.0	48	48	3/4	
2	10	0	—	—	—	10	16.3	21	—	—	
	10	120*	1	29	Daily	1	—	9	39	8/9	Few colonies
3	10	0	—	—	—	10	16.6	14	—	—	
	10	Glucose solution	—	—	5 times weekly	10	11.0	13	—	—	
	10	112	3	21	5 times weekly	7	—		59	3/3	3 colonies
Coccidioidomycosis											
4	10	0	—	—	—	10	8.5	9	—	—	
	10	400	2	48	5 times weekly	9	10.0	41	65	0/1	
	10	200	2	48	5 times weekly	3	17.0	19	65	7/7	
	10	100	2	48	5 times weekly	4	20.0	21	65	6/6	
5	10	0	—	—	—	4		34	40	6/6	
	10	240	1	29	Daily	1	—	9	40	5/9	6 of 20 tubes positive
6	10	0	—	—	—	10	13.4	17	—	—	
	10	Glucose solution	—	—	5 times weekly	10	12.5	17	—	—	
	10	112	3	21	5 times weekly	6		22	61	4/4	
7	10	0	—	—	—	9	16.8	27	47	1/1	
	10	55	3	21	3 times weekly	1	—	12	47	9/9	

* First two treatments at 240 mg. per kg.

The time of survival of treated mice after the death of control animals and the results of cultures of tissues of mice surviving to termination of the experiment (at least one week after termination of treatment) are the criteria by which efficacy of the drug is measured.

RESULTS

The *in vitro* activity of X-5079C is less remarkable than its therapeutic activity. *Histoplasma capsulatum*, *C. neoformans*, and *C. albicans*, after an interval of partial inhibition, grow in dilutions of 10 to 65 mg. of the drug per 1 ml. of broth. Narrow, faint zones of inhibition appeared around discs of filter paper impregnated with low dilutions of the antifungal agent placed on seeded agar plates. Even against the test strain of *P. varioti* supplied by Dr. Berger, X-5079C permitted germination of spores and some growth of mycelium, and it inhibited only production of aerial mycelium and conidia. This was apparently due to mycostatic rather than mycocidal properties of X-5079C, and to its decay in solution (3). By critical selection of culture medium, adjustment of the testing schedule to an optimum, and use of *P. varioti* as the test fungus, the disc test has been adjusted to an accurate bioassay of the drug in the serum of treated patients (4).

TABLE 3

RESPONSE OF MICE WITH EXPERIMENTAL HISTOPLASMOSIS TO TREATMENT WITH TWO LOTS OF X-5079C

Lot Number of X-5079C	Number of Mice	Drug Dose in Mg. per Kg.*	Delay before Therapy: Days	Duration of Therapy: Days	Schedule of Injections	Number of Deaths	Day of Median Death	Day of Last Death	Day Survivors Were Killed	Mice Positive/Mice Cultured
29	10	21.6	3	28	5 times weekly	0	—	No deaths	38	0/10
	10	10.8	3	28	5 times weekly	1	—	38	38	4/9
	10	5.4	3	28	5 times weekly	0	—	No deaths	39	9/10
	10	2.7	3	28	5 times weekly	4	10	26	39	6/6
	10	1.35	3	19	5 times weekly	10	10	20	None killed	—
41	10	21.6	3	28	5 times weekly	0	—	No deaths	38	0/10
	10	10.8	3	28	5 times weekly	0	—	No deaths	38	0/10
	10	5.4	3	28	5 times weekly	0	—	No deaths	38	1/10
	10	2.7	3	28	5 times weekly	1	—	11	38	6/9
	10	1.35	3	28	5 times weekly	7	11	17	38	3/3
Saline controls	20	None	3	—	5 times weekly	20	10	13	None killed	—
Untreated controls	10	None	—	—	—	9	10	10	38	1/1

* Doses in mg. per mouse per treatment were: 0.432, 0.216, 0.108, 0.054, and 0.027.

Toxicity Tests

In an acute toxicity test a single intraperitoneal injection of X-5079C (Lot No. 16) in a dosage of 625 mg. per kg. killed 8 of 15 mice within forty-eight hours after injection. The remaining 7 mice were killed on the fourth day after injection. Seven of the mice which died and 7 which were killed later were examined by Dr. L. L. Ashburn for histologic evidence of tissue damage.

Lot No. 16 of X-5079C is no longer available for experimental use, and lots prepared more recently, while equally potent, are much less toxic. The LD_m of Lot No. 29, administered in a single dose intraperitoneally to 17-gm. female white mice,[8] is more than 3,100 mg. per kg. The tissues of mice which received lethal and sublethal doses of this more highly purified preparation have not been examined. Studies of the histopathology in mice receiving Lot No. 16 of X-5079C are now of limited significance; therefore, Dr. Ashburn's report will be presented in summary rather than in detail.

In mice which died after the acute toxicity test, small droplets of lipid were found in parenchymal cells of the liver and in the epithelium of proximal convoluted tubules of the kidney. In a few cells in these areas, cytoplasmic oxyphilia and karyorrhexis were observed. An increase of lymphoid tissue in lymph nodes, spleen, and thymus, with karyorrhectic necrosis and phagocytosis of cellular debris, was apparent. Mice which survived this acute toxicity test and were killed four days later resembled control mice, except that depletion of lymphocytes and increased proliferation of large mononuclear cells were observed in two thymuses. The amount and distribution of lipid were normal.

Mice which were given 250 mg. per kg. of Lot No. 16 of X-5079C intraperitoneally survived and were killed four days later. In a few, minute lipid droplets in liver parenchymal cells and

other changes similar to those in mice given the larger dose of the antifungal agent were present. Many cells in mitosis were present in some animals. In mice that received an intravenous injection of 250 mg. per kg., the pathologic changes approached those induced by the higher dose given intraperitoneally.

In a chronic toxicity test, mice that received 100 mg. per kg. of Lot No. 16 of X-5079C intraperitoneally five times per week, to a total of thirty injections, survived. Ten mice were killed one day after the last injection, 5 at seven days, 5 at fourteen days, and 5 at seventy-six days. The only abnormalities seen in these mice were inflammatory changes in the mesentery, omentum, perirenal fat, the capsule and septa of the pancreas, and occasional small areas of the liver capsule and abdominal surface of the diaphragm. These inflammatory changes had almost completely disappeared in mice killed seventy-six days after the last injection.

Ten mice with experimental coccidioidomycosis, in an experiment in which all control animals died on the eighth and ninth days (an overwhelming infection) were given X-5079C (Lot No. 16) intraperitoneally at the level of 400 mg. per kg. five times per week (*experiment 4, see* table 2). This appeared to be a toxic dose in infected mice, but one mouse survived twenty-three treatments at this level, one survived twenty-six, and one survived thirty-five treatments. *Coccidioides immitis* was not isolated in culture from the last-mentioned mouse when it was killed fifty-six days after the last control mouse had died. There was no histologic examination of these mice. In another experiment, the antifungal agent was given intraperitoneally in a dosage of 240 mg. per kg. daily for twenty-nine consecutive days to mice with experimental histoplasmosis without clinical evidence of toxicity (*experiment 7, see* table 1).

In subsequent studies of this agent, other lots of drug have shown equal or higher potency and lower toxicity. Lot No. 29 of X-5079C has about the same potency as Lot No. 16, but is only one fifth as toxic. Intraperitoneal injection of 3,143 mg. per kg. killed only one of 3 mice. Lot. No. 30 is slightly more potent, and intraperitoneal injection of 3,333 mg. per kg. (60 mg. per mouse) killed only one of 7 mice. At this time histologic studies of mice receiving these less toxic lots are not available.

Experimental Therapy

The preparation X-5079C (Lot No. 16) was effective in preventing the death of mice with experimental histoplasmosis which were treated with only 5.3 mg. per kg. of the drug intraperitoneally for only seven days, although Histoplasma was isolated in culture from these mice when they were killed at termination of the experiment eleven days after the death of the last control mouse (*experiment 2, see* table 1). All 10 control mice in this experiment died between the eighth and fourteenth days post infection, indicating that this low dosage of the antifungal agent, administered over a short time, protected mice from an overwhelming infection, although it did not completely cure the infection. The severity of the test in this case was increased further by withholding treatment until the fourth postinfection day. The therapeutic activity under varying conditions of dosage, interval before beginning therapy, and duration of therapy are shown in the tables.

In table 3, lots Nos. 29 and 41 of X-5079C are compared and the efficacy of these lots at low levels is shown. Lot No. 41 prevented the death of 9 of 10 mice from experimental histoplasmosis when administered at 2.7 mg. per kg. in twenty treatments over a period of twenty-eight days, and sterilized tissues at 10.8 mg. per kg. The median day of death of 30 control mice in this experiment was day 10.

The antifungal agent is very effective in the treatment of experimental histoplasmosis in mice, partially effective in blastomycosis and coccidioidomycosis (*see* tables 1 and 2), and has no apparent effect upon the course of experimental cryptococcosis. It has been so reliable in its therapeutic action that long after its efficacy was firmly established it has been included as a standard of effective treatment in tests of other drugs. The tables, therefore, summarize only a few of the experiments in which this agent has been tested.

Discussion

It is apparent from the data presented above, part of which is summarized in the tables, that X-5079C has a remarkably low toxicity for mice, that moderately large doses of the drug can clear *H. capsulatum* from tissues of mice which have an otherwise overwhelming infection, and that very small doses (1/1,200 of the tolerated single dose) can prevent the death of mice from a dis-

ease which, without treatment, would have been rapidly fatal. Administration of the drug at a low dosage level can be delayed to the fourth day of an experimental disease which kills the last of the control animals on the fourteenth day (*experiment 2, see* table 1). Such an experiment simulates treatment of a naturally acquired, severe, established disease. The drug gives good, but partial, protection against coccidioidomycosis and blastomycosis, but is not effective against cryptococcosis.

The 1,200-fold margin between an effective daily dose of the drug in treatment of experimental mycoses in mice and a lethal dose is unique among drugs now available and effective in treatment of systemic mycoses.

The fact that viable Histoplasma cells persist for a month in mice on low dosages and short courses of X-5079C should not be interpreted as failure of the drug on these schedules. Man and animals exhibit strong tendencies to recover from histoplasmosis (and probably from all systemic mycoses), and any drug which protects mice from an experimental overwhelming infection as consistently as this agent does has obvious therapeutic potentialities for tipping the balance in favor of the host.

The therapeutic results of clinical trials and data on toxicity are reported in accompanying papers (5, 6). Regrettably, X-5079C still is not available in sufficient supply for fully adequate clinical evaluation.

Summary

A new antifungal agent, X-5079C, has an unusual spread between a minimal effective antimycotic dosage and maximal tolerated dosage. Its LD_{50} in acute toxicity tests in 16-gm. mice varied from 650 mg. per kg. in early lots to more than 3,300 mg. per kg. in 18-gm. mice in lots prepared recently. The most toxic lot tested was given in daily intraperitoneal injections of 240 mg. per kg. for one month without clinical evidence of toxicity. Survivors of a 625-mg. per kg. dosage of Lot No. 16 and mice on long-term therapy at high dosages of that lot did not show histologic evidence of permanent tissue damage.

The agent is effective in preventing death in mice with otherwise overwhelming experimental histoplasmosis, even when given at the dosage level of 5 mg. per kg. for as short a time as seven days. This dosage does not clear tissues of Histoplasma. At the level of 2.7 mg. per kg. given in twenty treatments over a period of twenty-eight days, Lot No. 41 of X-5079C protected 9 of 10 mice from death, and 10.8 mg. per kg. sterilized tissues. This therapeutic activity was achieved in an experiment in which the median day of death of 30 control mice was day 10.

The substance is effective against experimental histoplasmosis, partially effective against blastomycosis and coccidioidomycosis, and has no effect upon the course of experimental cryptococcosis.

Sumario

Actividad Quimioterapéutica y Tóxica del Nuevo Agente Antifungoso X-5079C en las Micosis Experimentales

Un nuevo agente antifungoso, el X-5079C, abarca una extensión inusitada entre una dosis antimicótica eficaz mínima y una dosis tolerada máxima. Su DL_{50} en ensayos de toxicidad aguda ejecutados en ratones de 16 gm., varió de 650 mg. por kg. de peso en los primeros lotes a más de 3,300 mg. por kg. de peso en ratones de 18 gm. en lotes preparados recientemente. El lote más tóxico ensayado fué administrado en inyecciones intraperitoneales diarias de 240 mg. por kg. de peso durante un mes sin signos clínicos de toxicidad. Los sobrevivientes de una dosis de 625 mg. por kg. de peso del Lote No. 16 y los ratones sometidos a terapéutica a largo plazo a dosis altas del mismo lote no revelaron signos histológicos de lesión permanente de los tejidos.

El agente resulta eficaz para impedir la muerte en ratones que tienen una histoplasmosis experimental que de otro modo sería agobiadora, aun cuando se administra a una dosis de 5 mg. por kg. de peso no más de siete días. Esta dosis no despeja los tejidos del histoplasma. A razón de 2.7 mg. por kg. de peso, administrado en veinte tratamientos durante un período de veintiocho días, el Lote No. 41 del X-5079C resguardó de la muerte a 9 de 10 ratones, y 10.8 mg. por kg. de peso esterilizaron los tejidos. Esta actividad terapéutica se obtuvo en un experimento que el día medio de la muerte de 30 ratones testigos fué el día 10.

La sustancia es eficaz contra la histoplasmosis experimental, parcialmente eficaz contra la blastomicosis y la coccidioidomicosis y no ejerce efecto alguno sobre la evolución de la criptococosis experimental.

Resume

Activité chimiothérapeutique et toxique du médicament antifungique X-5079C dans les mycoses expérimentales

Un nouveau médicament antifungique, X-5079C, a une étendue inhabituelle entre une dose

antifungique efficace minima et une dose maxima tolérée. Son LD_{50}, dans des tests d'intoxication aiguë chez des souris de 16 gm varie de 650 mg par kg dans les premiers lots, à plus de 3.300 mg par kg chez des souris de 18 gm dans les lots préparés récemment. On donna au lot testé le plus toxique des injections quotidiennes intrapéritonéales de 240 mg par kg, pendant un mois, sans évidence clinique de toxicité. Les survivants d'un dosage à 625 mg par kg du lot N° 16 et des souris ayant eu un traitement à long terme à haute dose, dans ce lot, ne montrèrent pas d'évidence histologique de dégat tissulaire permanent.

Le médicament est efficace dans la prévention de la mort de souris ayant une histoplasmose expérimentale qui serait autrement accablante, même si on le donne à la dose d'un ordre de 5 mg par kg pour une durée aussi courte que sept jours. Cette dose ne débarrasse pas les lésions d'Histoplasma. Avec la quantité de 2,7 mg par kg, donnée en vingt traitements pendant une période de vingt-huit jours, le lot N° 41 X-5079C protégea 9 des 10 souris de la mort, et 10, 8 mg par kg stérilisèrent les tissus.

Cette action thérapeutique fut accomplie dans une expérience dans laquelle le jour de la mort des souris de contrôle fut en moyenne le dixième jour.

Le produit est efficace vis à vis de l'histoplasmose expérimentale, partiellement efficace vis à vis de la blastomycose et de la coccidioidomycose, et n'a pas d'effet sur l'évolution de la cryptococcose expérimentale.

Acknowledgments

It is a pleasure to acknowledge the generosity of Hoffmann-La Roche, Inc., in making this antifungal agent available for study. During most of the four years since the drug's efficacy was clearly shown in its first experimental test in this laboratory, Willard R. Piggott, with the technical assistance of William Hill, has supervised the tests of X-5079C.

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CHEMOTHERAPEUTIC ACTIVITY OF X-5079C IN SYSTEMIC MYCOSES OF MAN[1, 2]

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INTRODUCTION

Twenty-eight systematic fungal infections in 27 patients have been treated with a new antifungal agent, X-5079C, following reports by Grunberg and associates (1) and Emmons (2) of its successful use in experimental mycoses in mice. The results reveal significant activity of the drug in cases of histoplasmosis, blastomycosis, aspergillosis, sporotrichosis, and maduromycosis. No benefit was observed in cases of coccidioidomycosis or Candida endocarditis. The present report describes this experience.

METHODS AND MATERIALS

Selection of cases: Patients at the Clinical Center of the National Institutes of Health with systemic fungal diseases were selected for the study. All of the patients were severely ill; in some instances their disease was in relapse following other treatment, and they were considered unlikely to recover spontaneously. Other aspects of some of these cases have been reported elsewhere (3–6) and patients are numbered identically in all reports. Final observations on the patients for the purpose of the present report were made in February, 1961.

Cultural methods: The diagnosis in each patient was corroborated by positive fungal cultures. Specimens of blood, bone marrow, urine, sputum, cerebrospinal fluid, sinus tract drainage; liver, lymph node, or other biopsy material; or oral swabs were promptly inoculated into culture media in the Clinical Pathology Laboratories of the Clinical Center. Brain–heart infusion agar (with and without rabbit blood) was used routinely. Occasionally penicillin, streptomycin, or chloramphenicol were added to the media when bacterial overgrowth was anticipated. In addition, all specimens were inoculated into trypticase soy broth and trypticase soy broth with blood. Colonies were picked and subcultured on Sabouraud dextrose agar. Identification was by gross and microscopic colonial morphology. Species of Candida were identified by their morphology and patterns of carbohydrate assimilation and fermentation. Cultures were repeated at regular intervals throughout the period of observation.

Clinical and laboratory studies: Usual laboratory procedures, roentgenograms, and several tests of hematologic, liver, and renal function were made during treatment to evaluate favorable or unfavorable effects of the drug. Criteria for evaluation of clinical response were established as follows:

Apparent recovery: failure to isolate the fungus in culture, disappearance of symptoms and signs of active infection, and no relapse during the period of observation.

Improved: objective improvement in clinical signs.

Unchanged: persistence of cultures positive for the organism and unaltered clinical state.

Preparation and administration of drug: The antifungal agent, X-5079C,[3] was received as a cream-colored powder and, to avoid lump or gum formation, it was dispersed by sprinkling into freshly prepared isotonic sodium chloride solution slightly less in volume than ten times the weight of the drug. Mixing was continued with a magnetic stirrer without heating until the solution appeared clear. It was then transferred to a sterile graduate and made up to a final concentration of 1 gm. per 10 ml. by the addition of saline solution. It was further clarified by passage serially through chemically clean millipore membranes of 5, 3, and 1.5 μ in size. Sterilization was by filtration through a sterile size-6 Seitz filter, after which the drug was transferred under ultraviolet light to rubber-stoppered B corkage vials in 10-ml. amounts. United States Pharmocopeia sterility tests employing thioglycollate and Sabouraud media were performed, and the final product, a clear amber solution, was kept thereafter at 4°C.

The drug was administered subcutaneously every six hours with a 25-gauge five-eighths-inch needle.

Dosage used: On the basis of studies of experimental infection in animals, a total daily dosage of 5 mg. per kg. was initially selected. In a few patients who were not responding to treatment, dosage was increased to 9 to 17 mg. per kg. Three to 5 mg. per kg. per day were selected as the dosage for the remaining patients.

[1] From the Infectious Disease Service, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases; and the Laboratory of Infectious Diseases, Medical Mycology Section, National Institute of Allergy and Infectious Diseases, Bethesda 14, Maryland.

[2] Presented in part before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, on May 22, 1961.

[3] The X-5079C used in this study was furnished by Hoffmann-LaRoche, Nutley, New Jersey.

Results

Histoplasmosis

X-5079C was used in 8 patients with histoplasmosis (table 1). Five of these had severe disseminated infection and 3 had chronic cavitary, pulmonary disease. Three patients have apparently recovered, 3 are improved, and 2 are unchanged. *Patient 39,* considered "unchanged" received the drug for a period of time (six days) too short to evaluate its therapeutic effectiveness. *Patients 37* and *88* had persistently positive cultures of some specimens despite therapy, suggesting that the severe disseminated form of disease is more resistant to therapy than chronic pulmonary forms.

Case Reports

Patient 27, O.B., a 75-year-old white lawyer, became ill in November, 1957, with cough, fever, easy fatigue, and anorexia, and never thereafter regained his former state of health. In June, 1958, a painful ulcer developed and spread along the lower left dentage ridge to involve eventually the anterior and posterior pillars, posterior pharynx, and larynx. The lesion was covered by a necrotic, hemorrhagic crust. Hoarseness and marked dysphagia resulted. Smears and cultures of lesions showed *Histoplasma capsulatum*. On admission to the Clinical Center on October 22, 1958, the patient was markedly cachectic, disoriented, stuporous, hypotensive, febrile, dehydrated, and oliguric. Hepatosplenomegaly and pedal edema were present. A chest film revealed a 5- by 10-mm. nodular density in the third anterior interspace. The erythrocyte sedimentation rate was elevated to a maximum of 120 mm. per hour, and the hemoglobin was 9.9 gm. per 100 ml. Serum complement-fixation and histoplasmin skin tests were negative. The blood urea nitrogen was 129; serum globulin, 4.4 mg. per 100 ml.; potassium, 7.5; and carbon dioxide content, 9 mEq. per liter. Repeated blood, urine, oral swab specimens, and a bone marrow aspirate were positive on culture for *H. capsulatum*. Microscopic study of liver and oral mucous membrane biopsies showed granulomatous inflammation in both, and organisms suggestive of *H. capsulatum* in the latter. On X-5079C and supportive treatment, the patient improved markedly; and cultures of blood, bone marrow, and swabs of oral lesions became negative. Urine cultures continued to be positive. The blood urea nitrogen and electrolytes returned to normal. Therapy was interrupted to allow observation on the nature of side effects of the drug. Clinical improvement continued and urine cultures became negative on the institution of amphotericin treatment. Hydrocortisone in a dosage of 25 to 50 mg. per day was given also because of hypotension, hyperkalemia, and hyponatremia. The patient died with an intercurrent, fulminating necrotizing staphylococcal bronchopneumonia and at autopsy had massive caseous lesions of the adrenal due to *H. capsulatum*.

Patient 37, S.R., a 44-year-old male painter, in 1952 developed a chronic meningitis manifested by increased lymphocytes, elevated protein, and depressed glucose in the cerebrospinal fluid. He was studied at the Clinical Center during six admissions, beginning on November 15, 1957. Repeated cerebrospinal fluid, urine, sputum, and bone marrow cultures were consistently negative for fungi until cerebrospinal fluid removed on March 17, 1959, at pneumoencephalography was positive for *H. capsulatum*. Histoplasmin skin and complement-fixation tests in serum dilution of 1:128 with histoplasmin and blastomyces antigen were positive. Prior to therapy with X-5079C, cultures of bone marrow, lymph node, urine, and sputum were still negative, as was culture of cerebrospinal fluid of April 8, 1959. After twenty-two days of therapy with the antifungal agent, no improvement attributable to the drug was observed. Repeated cerebrospinal fluid examinations revealed an additional positive culture of fluid on June 24, 1959. Amphotericin therapy from May 28 to July 3, 1959 (total dosage 1,255 mg.) was accompanied by a slight improvement in cerebrospinal fluid findings with a decrease in lymphocytes and protein and an increase in glucose; but abnormal findings in cerebrospinal fluid continued.

Patient 46, C.B., a 66-year-old white farmer, became ill in 1956 with dyspnea, cough, weight loss, easy fatigue, and anorexia. A chest film revealed right upper lobe disease and, after lobectomy and thoracoplasty on July 5, 1957, microscopic study of resected tissue showed organisms suggestive of *H. capsulatum*. On the patient's admission to the Clinical Center on July 2, 1959, bronchial breathing was heard throughout the lungs, and extensive bilateral fibrotic changes were seen on the chest film. Laboratory studies revealed polycythemia, a positive histoplasmin skin test, and whole yeast phase and histoplasmin complement-fixation tests in serum dilution of 1:32. Repeated sputum cultures were positive, but urine and bone marrow were negative for *H. capsulatum*. Postural drainage and tetracycline therapy were attended by lessened dyspnea and sputum production, but no change in positive fungal cultures. Therapy with X-5079C from August 10 to September 10, 1959, was followed by disappearance of *H. capsulatum* from all sputum specimens collected subsequent to August 21 and by disappearance of polycythemia. Five months post antifungal therapy, sputum specimens were again positive for *A. fumigatus*. A corresponding increase in dyspnea and in sputum production with blood-tinging occurred. On reinstitution of anti-

TABLE 1

HISTOPLASMOSIS: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Previous Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg. (mg./kg.)*		
27	O.B.	75	Severe, disseminated	None	Blood, bone marrow, oral ulcer, urine	10/23/58–11/17/58	3,700	400 (9)	Negative	Improved; treated subsequently with amphotericin
									Positive	Died
37	S.R.	44	Severe, disseminated (meningeal)	None	Cerebrospinal fluid	4/14/59–5/12/59	4,050	200 (3)	Positive	No change; treated subsequently with amphotericin
46	C.B.	66	Chronic, cavitary, pulmonary	None	Sputum	8/16/59–9/11/59	5,300	200 (4)	Negative	Apparent recovery
76	S.C.	71	Chronic, cavitary, pulmonary	None	Sputum	5/26/60–6/22/60	6,160	220 (4)	Negative	Apparent recovery
73	C.B.	44	Chronic, cavitary, pulmonary	None	Sputum	6/6/60–7/4/60	6,720	240 (4)	Negative	Apparent recovery
39	C.D.	17	Severe, disseminated	Amphotericin	Pleural fluid;* lymph node*	8/11/60–8/16/60	700	140 (4)	Unchanged	Unchanged
88	B.A.	63	Severe, disseminated	None	Blood, bone marrow, sputum, urine				Negative	
					Skin and epiglottic biopsy	9/17/60–10/29/60	12,840	240 (4)	Positive	Improved
					Liver biopsy				Not performed	
			(Asymptomatic)		Epiglottic biopsy	11/7/60–11/28/60	6,300	300 (4)	Negative	Unchanged
					Skin biopsy				Positive	
			Pharyngeal ulcer		Pharyngeal ulcer sputum, urine	1/13/61–		240 (3)	Pending	Improved
99	O.G.	49	Severe, disseminated	None	Blood, bone marrow, lip lesion, urine, sputum	12/21/60–1/10/61	4,800	240 (4)	Pending	Improved

* Cultures negative immediately prior to treatment.

fungal therapy, clinical improvement occurred, but cultures remained positive.

Patient 76, S.C., a 71-year-old white farmer, had roentgenographic evidence of stable, left upper lobe disease known since 1947, followed annually from 1949 to 1956, and ascribed to tuberculous infection presumably acquired from exposure to his mother who died of this disease in 1907. In October, 1959, he noted fatigue, anorexia, an 18-pound weight loss, and nonproductive cough. A chest film obtained on December 18, 1959, revealed old disease and, in addition, small cavities in the right upper lobe and diffuse infiltration with fibrosis in the right lower lobe adjacent to the mediastinum. In the local hospital in January, 1960, and at the Clinical Center in April, 1960, sputum cultures were positive for *H. capsulatum*. A histoplasmin skin test was positive, as was the whole yeast phase antigen complement-fixation test in serum dilution of 1:32. Therapy with X-5079C was associated with negative cultures, a fall in titer of the complement-fixation test to 1:8, a weight gain of 5 pounds, and subjective improvement in fatigue, cough, and anorexia. No change was observed in the chest films. Improvement had continued at the time of the visit in December, 1960.

Patient 73, C.B., a 44-year-old white male civil servant, resident of the Panama Canal Zone, had had known, right-sided, chronic pulmonary disease since 1954, diagnosed histopathologically as histoplasmosis in December, 1959, following thoracotomy and lobectomy. Early in March, 1960, he noted increasing dyspnea, a weight loss of 10 pounds, and a chest film indicated spread of disease to the left lung. On admission to the Clinical Center on March 19 he appeared chronically ill, and a chest film showed bilateral fibrotic-appearing upper lobe disease. A histoplasmin skin test was negative, as were the complement-fixation tests. Repeated sputum cultures were positive for *H. capsulatum*. Cultures became negative and there was objective improvement after therapy with X-5079C.

Patient 39, C.D., a 17-year-old school boy, had known cirrhosis, hypogammaglobulinemia, and histoplasmosis based on positive cultures of lymph node and pleural fluid in January, 1959. From April 8 to June 6, 1959, he received a total of 1.1 gm. of amphotericin. He was able to return to school thereafter, although he still had physical and chemical findings of moderately severe cirrhosis. At the time when he returned for re-evaluation in July, 1960, a marked increase in lymphadenopathy was noted, and biopsy of a supraclavicular node revealed organisms typical of *H. capsulatum*. Histoplasma complement-fixation tests were consistently negative (possibly on the basis of hypogammaglobulinemia) as were histoplasmin skin tests. Pending cultural studies of the lymph node (which were negative) he received one week's treatment with X-5079C. No therapeutic effect was detected during this short course of therapy.

Patient 88, B.A., a 63-year-old white male farmer, had known Hodgkins disease, diagnosed histologically in 1958 but quiescent thereafter. In April, 1960, he noted progressive malaise, lethargy, anorexia, cough, and hemoptysis. In May, 1960, a diagnosis of pulmonary tuberculosis was made and confirmed by two sputum cultures positive for *M. tuberculosis*. He was placed on isoniazid and aminosalicylic acid (PAS) therapy. Cultures became negative and he improved temporarily. In early August, progressive dysphagia and sore throat developed, and at the time of admission he was not able to swallow solid food or tablets. He lost 49 pounds. On admission to the Clinical Center on September 2, the patient was emaciated, febrile, and there were multiple white plaques with raised edges and central ulceration on the soft palate. A granular hypertrophic lesion was seen on the epiglottis. On the skin of the back there were about 12 brownish papules measuring 0.5 to 1.0 cm. in diameter. Cultures of blood, bone marrow, sputum, and urine were positive for *H. capsulatum*, as were skin, palatal, epiglottic, and liver biopsies. A histoplasmin skin test was negative, and the yeast phase complement-fixation test was positive in serum dilution of 1:32. Therapy with X-5079C was associated with rapid lessening of dysphagia detectable within 48 hours of starting the drug. Cultures of all specimens became negative save the repeated epiglottic lesion biopsy. The patient regained 40 pounds. He received a second course of therapy, but six weeks after this he developed dysphagia and had cultures from urine, sputum, epiglottic and skin biopsy lesions positive for *H. capsulatum*. He is currently on a third course of therapy.

Patient 99, O.G., a 49-year-old white, male truck mechanic, became ill in August, 1960, with night sweats, fever, easy fatigue, a 10-pound weight loss, and dyspnea. A grade III aortic diastolic murmur with thrill, peripheral signs of aortic regurgitation, an ulcerative lesion of the lip, and a pulsatile mass (representing an abdominal aneurysm) were found on physical examination. Cultures of blood, bone marrow, urine, lip lesion, and sputum were positive for *H. capsulatum*. A histoplasmin skin test was negative, and the complement-fixation test was positive (histoplasmin 1:512, whole yeast phase antigen 1:128). Renal function was impaired, with blood urea nitrogen of 51 mg. and creatinine 3.5 mg. per 100 ml. of blood. The kidneys were not observed on the intravenous pyelogram. Despite the absence of signs of peripheral emboli and because he had changing murmurs, the patient was treated as a case of Histoplasma endocarditis. Therapy with X-5079C was associated with marked clinical improvement. Sputum cultures remained positive, however, during the course of therapy which was of suboptimal duration. Further therapy is planned.

Blastomycosis

Five patients with blastomycosis have made apparent recoveries from their disease and one

patient improved remarkably (table 2). Improvement occurred whether the infection was clinically disseminated to the skin or to subcutaneous tissue (*Patients 35, 94*) or was more widespread to involve bone (*Patients 19, 65*), lung and kidneys (*Patient 83*), or was still localized clinically to the lungs (*Patient 11*). *Patient 94*, who was unresponsive and critically ill on admission, is of special interest since he has recovered almost completely from his illness, but still has *B. dermatitidis* in histologic sections and cultures of the residual, fibrotic lesions of his subcutaneous tissue. The clinical course of *Patient 11* is shown in figure 1.

Case Reports

Patient 11, D.R., a 72-year-old Negro housewife, developed a four-week illness characterized by cough, hoarseness, and sore throat while visiting in Grifton, North Carolina, in the summer of 1953. Later that year pleuritic right chest pain began and a chest film revealed right upper lobe disease. In March, 1955, the histopathologic diagnosis of blastomycosis was made after thoracotomy and resection of the right upper lobe. In January, 1956, hemoptysis occurred; and on February 3, 1956, the patient was first admitted to the Clinical Center where sputum cultures were positive for *Blastomyces dermatitidis*. In November and December, 1956, she received a total of 6.050 gm. of 2-hydroxystilbamidine without change in the cultural status. In May, 1957, she received 110 mg. of amphotericin intravenously over an eleven-day period. The patient interrupted therapy and because of the side effects could not be persuaded to resume treatment. Cultures became negative for two months. She continued thereafter to be asymptomatic except for morning cough productive of a smal amount of sputum positive by culture for *B. dermatitidis*. Blastomyces complement-fixation and blastomycin skin tests remained negative. After two weeks of a four-week course of X-5079C, cultures became negative and remained so.

Patient 19, W.O., a 20-year-old Negro male dishwasher, became ill in the fall of 1957 with pyuria, bilateral flank pain, and a weight loss of 20 pounds. In January, 1958, a retroperitoneal mass was demonstrated on intravenous pyelography, and surgical exploration revealed a psoas abscess. Cultures of purulent drainage material showed *B. dermatitidis*. Osteolytic lesions were seen later in lumbar vertebrae 2, 3, and 4. On admission to the Clinical Center on March 6, 1958, the patient appeared wasted, chronically ill, and had a draining sinus in the right anterior flank. Exploration of the area on March 28, 1958, revealed a right psoas

TABLE 2

BLASTOMYCOSIS: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Previous Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg. (mg./kg.)*		
11	D.R.	72	Chronic, pulmonary	Hydroxystilbamidine and amphotericin (relapse)	Sputum	1/15/60–2/12/60	5,700	200 (3)	Negative	Apparent recovery
19	W.O.	20	Osteomyelitis, cellulitis	Amphotericin (relapse)	Sinus tract drainage	2/17/59–4/2/59	15,200	400 (6)	Negative	Apparent recovery
35	W.B.	57	Skin	Potassium iodide	Skin	2/14/59–3/4/59	4,650	400 (5)	Negative	Apparent recovery
65	A.W.	40	Osteomyelitis, cellulitis, pulmonary	None	Sinus tract drainage	10/12/59–11/20/59	10,060	280 (4)	Negative	Improved
									Positive	Relapsed 2 months after treatment.
						2/29/60–5/4/60	18,680	280 (4)	Negative	Apparent recovery
83	O.E.	47	Pulmonary, renal, skin	None	Skin, sputum, urine	6/24/60–8/18/60	11,050	200 (4)	Negative	Apparent recovery
94	C.T.	70	Pulmonary, cellulitis, prostate	Amphotericin	Subcutaneous abscesses	10/22/60–12/3/60	8,195	200 (4)	Positive	Improved

abscess extending across the midline at the third lumbar vertebra. The erythrocyte sedimentation rate was elevated to 121 mm. per hour, hemoglobin was 11.5 mg. per 100 ml., and the peripheral leukocyte count was 17,800 mm.^{3}. The blastomycin skin test and Blastomyces complement-fixation test were negative. Cultures of sputum and sinus tract drainage were positive for *B. dermatitidis*. Lung fields were normal roentgenographically. The patient's spine was immobilized with a plaster cast, and from March 14 to June 10 he received 2,298 mg. of amphotericin. On this therapy the cultures became negative, and he was discharged, ambulatory, and much improved.

On February 1, 1959, on readmission, an elevated temperature was recorded, and within two weeks a tender mass was noted at the medial margin of the operative scar. Purulent fluid positive for *B. dermatitidis* was obtained on incision of this mass. On therapy with X-5079C fever disappeared and the cultures became negative. The patient was discharged, ambulatory, and has had no recurrence of illness.

Patient 35, W.B., a 57-year-old white logger, in June, 1957, noted the appearance of a tender area inside his left nostril which over the next nineteen months slowly enlarged to involve the upper lip, ala, and mucous membrane of the nose. Potassium iodide therapy was ineffective. On the patient's admission to the Clinical Center on January 12, 1959, the lesion measured 5 cm. in diameter, was verrucous, raised, fissured, and had minimal purulent drainage. Blastomyces complement-fixation and blastomycin skin tests were negative. Organisms typical of *B. dermatitidis* were seen on microscopic study of biopsy material, and cultures of the lesion were positive. Therapy was accompanied by negative cultures and gradual disappearance of the lesion with minimal residual scarring.

Patient 65, A.W., a 40-year-old white male textile factory worker, in July, 1959, noted the onset of lower back pain, especially in the left lumbar area, increasing in severity until, in August, he had to quit working. In September, 1959, a tender, painful mass appeared over the left shin at the site of an old previously well-healed ax injury. On admission to the Clinical Center on September 23, 1959, a diffuse, subcutaneous, tender, slightly fluctuant swelling measuring 25 by 10 cm. was observed in the left lumbar area. Over the left midtibia was an indurated, reddish-purple swelling, 12 cm. in diameter, with a recent draining incisional scar. Cultures of drainage material from this site and from the lumbar area were positive for *B. dermatitidis*. Cultures of blood, sputum, bronchial washings, bone marrow, and urine were negative. Blastomyces complement-fixation and blastomycin skin tests were negative. The erythrocyte sedimentation rate was 130 mm. per hour, and serum globulins were 4.4 gm. per 100 ml. A chest film showed an infiltration in the right upper lobe; a film of the left tibia showed an osteomyelitic lesion measuring 3 cm. in greatest diameter; and lumbar spine films showed osteomyelitis of lumbar vertebrae 1 and 2.

On therapy with X-5079C, cultures became negative and the lesion of the left tibia area healed. With supplemental incision and drainage the lumbar lesion similarly resolved. The pulmonary infiltration disappeared. The erythrocyte sedimentation rate fell to 48 mm. per hour, serum globulins to 3.1 gm. per 100 ml., and the peripheral leukocyte count to 7,300 per mm.^{3}. There was little change in tibial films, but lumbar vertebral lesions improved. Following discharge on December 11, the patient felt well until approximately January 23, 1960, when back pains recurred. On January 26 spontaneous drainage began at the site of the old lumbar incision. Culture of drainage material from this site was again positive for *B. dermatitidis*. The tibial lesion was unchanged. The erythrocyte sedimentation rate was again elevated (103 mm. per hour), the leukocyte count was 18,000 per mm.^{3}, and globulin was 4.5 gm. per 100 ml. Incision and drainage and antifungal therapy again resulted in marked improvement in clinical status, the laboratory values given above, and in negative cultures.

Patient 83, O.E., a 47-year-old Negro male farmer, in February, 1960, began to cough, with production of greenish-yellow, blood-flecked sputum. In April, 1960, he first noted skin lesions of the right ankle and subsequently of the left and right face, occiput, left upper arm, right thumb, and left fifth finger. The lesions were considered typical of those of blastomycosis and, after preliminary treatment with potassium iodide, the patient was admitted to the Clinical Center on June 16, 1960. Cultures of the skin, sputum, and urine were repeatedly positive for *B. dermatitidis*. A blastomycin skin test was positive as was the Blastomyces complement-fixation test in serum dilution of 1:64. The erythrocyte sedimentation rate and serum globulins were elevated and the chest film showed disseminated, nodular, fibrotic lung lesions. Improvement, discernible within three days of starting X-5079C, continued, with complete disappearance of all skin lesions without typical scarring at the end of therapy. Cultures became negative, the erythrocyte sedimentation rate and serum globulin returned to normal, the complement-fixation titer fell to 1:16, and the patient gained 9 pounds. The chest film was unchanged. He appeared well in December, 1960, at the time of a return visit to the Center.

Patient 94, C.T., a 70-year-old white farmer, in July, 1960, first noted easy fatigue, progressive weakness, weight loss, dyspnea, and pyuria; in September he observed red, indurated, subcutaneous nodules. Aspiration of one of these revealed pus positive on culture for *B. dermatitidis*. He seemed to become more ill rapidly and was transferred to the Clinical Center in oxygen, in congestive failure, disoriented, confused, respon-

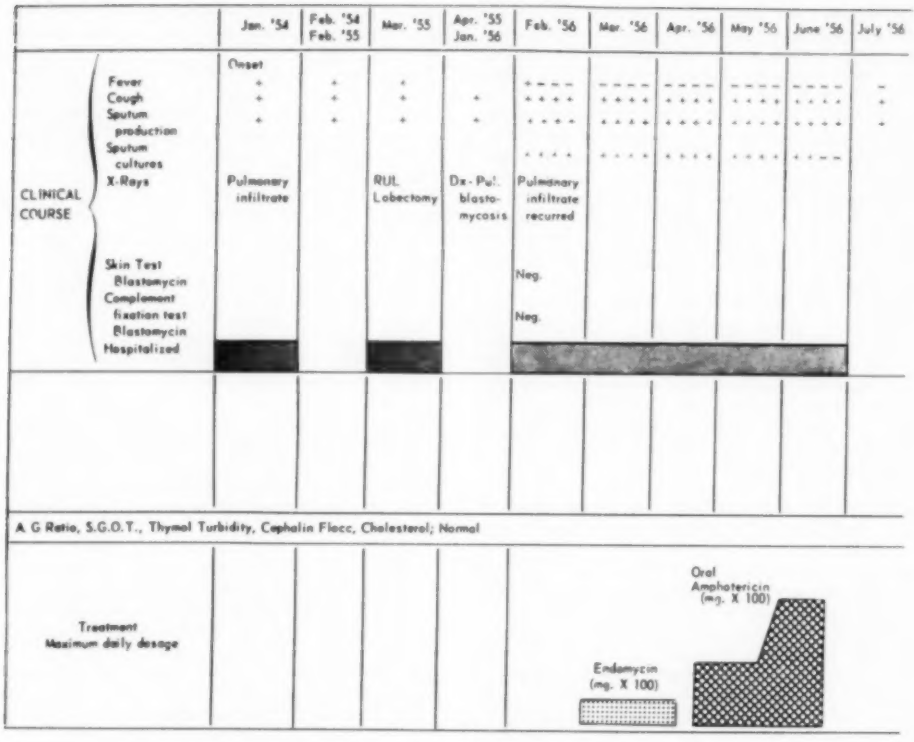


FIG. 1. *Patient 11* with blastomycosis.

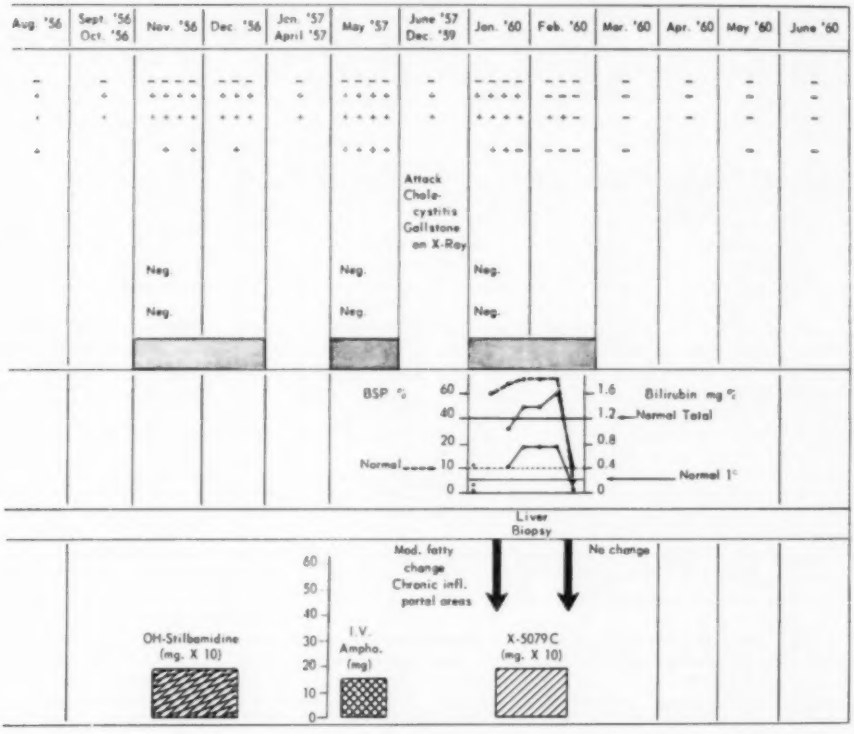
sive only to painful stimuli, dehydrated, and emaciated. Cultures of additional subcutaneous abscesses present in great numbers on both lower and upper extremities revealed Blastomeyes; but urine, blood, sputum, and bone marrow cultures were negative. A blastomycin skin test was negative and the Blastomyces complement-fixation test was positive in serum dilution of 1:8. A chest film revealed extensive diffuse, infiltrative, reticular changes. Therapy with X-5079C was associated with dramatic improvement so that the patient was able to leave bed and be up and about the nursing unit. Lesions of the subcutaneous tissues disappeared, leaving minute, depressed nodular scars. Culture of one of these after conclusion of therapy revealed Blastomyces. Transurethral resection of the prostate performed after treatment on December 29, 1960, showed organisms morphologically consistent with *B. dermatitidis*, but cultures of this tissue were negative. Although the patient is asymptomatic and ambulatory, further therapy is envisioned because of positive cultures, elevated erythrocyte sedimentation rate, and residual pulmonary findings.

Aspergillosis

In *Patient 43* with *Aspergillus fumigatus* infection disseminated from the lungs to subcutaneous tissues, prostate, and probably kidneys, marked clinical improvement occurred and cultures became negative on treatment. However, cultures again became positive eight and nine weeks after completion of the two treatment periods. The disease in *Patient 46* was unchanged. In the latter patient biopsies of lung or bronchi were not performed, and tissue invasion was not proved (table 3). (The case report of *Patient 46* appears in the section on Histoplasmosis.)

Case Report

Patient 43, K.H., a 27-year-old white male clerk, in 1951 developed progressive dyspnea, chronic cough, and diffuse pulmonary infiltrations, as



(FIG. 1, *continued*)

shown on a chest film. Cultures of sputum, including a number at the National Institutes of Health at that time, repeatedly showed *Aspergillus fumigatus*. No antifungal therapy was attempted, and he was able to work until July, 1959. On admission to the Clinical Center on July 23, 1959, the patient appeared dyspneic and chronically ill. The right epididymus was tender, and the prostate was enlarged, tender, and boggy. A chest film and tomography showed extensive chronic inflammatory lung disease with bilateral cavitation. Sputum and urine cultures were consistently positive, but blood and bone marrow were negative for *A. fumigatus*. During his stay at the Clinical Center, small hemoptyses occurred frequently, and severe hemoptysis requiring transfusions occurred once. Shortly after admission, marked edema of the shaft and glans of penis developed followed by a profusion of 1- to 2-mm. cutaneous and subcutaneous abscesses, needle punctures of which revealed *A. fumigatus* on culture. Peripheral leukocytosis, an erythrocyte sedimentation rate of 100 mm. per hour, and serum globulins of 5.6 gm. per 100 ml. were present. Therapy with X-5079C for three weeks was accompanied by remarkable clinical improvement and conversion of urine and sputum cultures to negative. The erythrocyte sedimentation rate and serum globulins decreased and leukocytosis disappeared. Eight weeks after stopping therapy, sputum was again positive for *A. fumigatus*, but the clinical status remained much improved. A second, six-week course of therapy with the antifungal agent again resulted in negative cultures for a subsequent seven-week period.

Sporotrichosis

Two patients (*Patients 71* and *74*) with severe *S. schenckii* infection involving bone and joints improved with treatment. In *Patient 71* cultures became negative, whereas in *Patient 74* cultures were negative prior to starting therapy despite marked progression of disease during periods of observation. Both patients have residual joint deformity. The third, *Patient 87*,

TABLE 3

ASPERGILLOSIS: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Previous Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg. (mg./kg.)*		
43	K.H.	27	Severe, disseminated	None	Sputum, urine, subcutaneous abscesses	8/28/59–9/17/59	5,050	250 (4)	Negative	Improved
									Positive	Cultural relapse 8 weeks after stopping treatment
						12/15/59–1/22/60	10,010	260 (4)	Negative	
									Positive	Cultural relapse 7 weeks after stopping treatment
46	C.B.	66	Pulmonary	*See* table 1	Sputum	3/10/60–4/26/60	9,900	220 (4)	Positive	Unchanged

TABLE 4

SPOROTRICHOSIS: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Previous Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg.(mg./kg.)*		
71	H.L.	56	Synovitis and osteomyelitis	Iodides	Subcutaneous tissue and bone	2/23/60–3/31/60	9,685	260 (4)	Negative	Improved
74	M.H.	31	Synovitis and osteomyelitis	Iodides and amphotericin	Subcutaneous* tissue	5/25/60–6/23/60	6,100	200 (4)	Negative	Improved
87	O.B.	37	Synovitis	Iodides	Synovial fluid	9/29/60–10/26/60	6,720	240 (4)	Positive	Improved relapsed

* Cultures negative immediately prior to treatment.

improved but the disease relapsed, positive cultures following treatment (table 4).

Case Reports

Patient 74, M.H., a 31-year-old white housewife, noted slowly progressive stiffness and aching in the right elbow in June, 1957. In the fall of that year he developed signs and symptoms of nephrosis, and a histopathologic diagnosis was made of nephrotic glomerulonephritis. This disease improved with corticosteroid therapy. Biopsy of the elbow was performed in July, 1959, and microscopic studies of tissue suggested—and cultures confirmed—the diagnosis of sporotrichosis. On admission to the Clinical Center on January 18, 1960, physical examination was negative save for the right elbow, which was swollen, reddened, fixed at 90° of flexion, and had a 1-cm. postoperative draining sinus. Elbow-joint film showed soft tissue swelling, synovial effusion, loss of joint space, and osteomyelitic changes of adjacent bone. Serum contained agglutinating antibodies for *S. schenckii* in a dilution of 1:1,280. Tissues obtained at surgery were positive on culture. Synovectomy of the right elbow joint, resection of the diseased bone, and application of a plaster cast were performed. Therapy was initiated with X-5079C with healing of the draining wound, decreased pain and swelling, clearing of infection, and a fall to 1:80 of agglutinating antibody titer.

Patient 74, M.H., a 22-year-old white housewife, was found to have *S. schenckii* on histologic sections and culture of a supraclavicular lymph node in August, 1959, at a time when she was hospitalized because of weakness, malaise, weight loss, and anemia. Potassium iodide in a dosage of 40 drops three times daily did not prevent spread of disease to the right elbow, knee, and left ankle. *S. schenckii* was cultured from sinus tract drainage from the right elbow. In January and February, 1960, she received 939 mg. of amphotericin and

was referred to the Clinical Center. Roentgenograms showed osteolytic lesions in the right radius, ulna, and distal left fibula, and linear and nodular densities with extensive pleural changes in the left lung field. The erythrocyte sedimentation rate and serum globulin were markedly elevated. The *S. schenckii* agglutination titer was 1:160, but cultures were negative. During a three-month period of observation and supportive therapy only, the disease progressed with enlargement and extension of the draining right elbow lesion. Prompt improvement occurred on therapy with X-5079C with closure of the draining lesion of the right elbow and reossification of lesions of the radius and ulna. The erythrocyte sedimentation rate fell moderately, but the serum globulins remained elevated. The patient was able to leave her bed and regained her ability to walk. On re-examination in November, 1960, she had continued to improve. However, her right knee was still warm and swollen and the *S. schenckii* titer had risen to 1:640.

Patient 87, O.B., a 37-year-old white female florist, who had worked continuously with flowers (and particularly roses), but who had been well and remembered no associated trauma, developed pain, tenderness, swelling, and redness of her right knee in June, 1959. She had a weight loss of 33 pounds and night sweats, but no fever. A short suboptimal course of iodide therapy was not associated with improvement. On admission to the Clinical Center on September 9, 1960, the right knee was warm, red, tender on movement, lacked 15° of complete extension, and had synovial thickening without detectable effusion. She was unable to walk. The erythrocyte sedimentation rate was elevated and the *S. schenckii* agglutination titer was 1:640. Physical therapy and treatment with X-5079C was associated with objective improvement in the knee findings, and the patient was able to walk at the time of discharge on November 5, 1960. A culture of aspirate of the knee was still positive, however, on November 3, and the agglutination titer and erythrocyte sedimentation rate were unchanged. In mid-January, 1961, swelling and pain recurred and she was considered to have had a relapse of the disease at readmission on January 30.

Maduromycosis

Patient 31 had an atypical *Madurella grisea* infection of the foot of two years' duration. He had failed to respond to three courses of amphotericin treatment, but the lesion became sterile and draining sinuses closed after antifungal therapy (table 5).

Case Report

Patient 31, C.P., a 61-year-old white farmer, in the fall of 1957 inadvertently scratched his left leg with a reed and developed an ulcerative lesion at the site. For a time some confusion existed as to the etiology of the infection, and for this and for an infiltration in his lung he received 3.375 gm. of 2-hydroxystilbamidine. Confusion was partially resolved in March, 1958, when *Cryptococcus neoformans* was cultured from the cerebrospinal fluid and another unidentified fungus was cultured from the ankle lesion. For these infections the patient then received four weeks' treatment with amphotericin. After clinical relapse and positive cultures he was re-treated with amphotericin from June to August and again in November, 1958. On admission to the Clinical Center on February 1, 1959, the patient appeared confused and disoriented. A poorly demarcated area of discolored, nodular, indurated, crusted lesions was seen in the skin and subcutaneous tissue of the left ankle. Microscopic study of biopsy tissue from the area showed focal granulomatous inflammation with necrosis and pigmented fungi. Cultures of swabs and biopsy tissue revealed *Madurella grisea*. Chronic bilateral inflammatory disease was present on the chest film. Cerebrospinal fluid contained many lymphocytes, decreased glucose, increased protein, and *C. neoformans* on culture. Re-treatment in February and March, 1959, with amphotericin (1,551 mg. intravenously and 9.5 mg. intrathecally) improved his clinical status, cerebrospinal fluid findings, and "converted" cerebrospinal fluid cultures to negative. No change was noted in the ankle lesion, however, and cultures continued to be positive for *M. grisea*. After twenty days' treatment with X-5079C, cultures became negative and the lesion cleared with residual scarring.

Coccidioidomycosis

Three patients with chronic pulmonary, and 2 patients with disseminated, coccidioidomycosis failed to respond to therapy. In one patient

TABLE 5

MADURELLA GRISEA INFECTION: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Previous Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg.(mg./kg.)*		
31	C.P.	61	Cellulitis	Amphotericin	Sinus tract drainage	8/21/59–8/30/59	2,100	250 (3.5)		
						10/14/59–11/2/59	5,700	300 (4)	Negative	Apparent recovery

TABLE 6

COCCIDIOIDOMYCOSIS: TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Pevious Treatment	Sites of Positive Cultures	Dates of Treatment	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg.(mg./kg.)*		
14	A.J.	82	Chronic pulmonary, with bronchopleural fistula	Amphotericin	Sputum	5/26/58–6/1/58	4,200	1,000 (17)	Positive	Dead
21	J.F.	25	Chronic pulmonary, synovitis, cellulitis, and osteomyelitis	Amphotericin	Sinus tract drainage	6/23/58–6/29/58	2,400	400 (8)	Negative	Improved; treatment followed, however, by amphotericin; relapsed 6 months after therapy during pregnancy
						11/27/59–1/21/60	11,100	200 (4)	Positive	Unchanged
33	P.G.	23	Chronic pulmonary and cerebellar abscess	Amphotericin	Sputum, cerebrospinal fluid, brain tissue	5/23/59–6/4/59	4,500	400 (7)	Positive	Dead
66	T.M.	52	Chronic pulmonary	Amphotericin	Sputum	1/24/60–4/3/60	11,240	160 (3)	Positive	Unchanged
93	H.E.	41	Chronic pulmonary	None	Sputum	10/21/60–11/14/60	15,180	700 (10)	Positive	Unchanged
97	H.F.	32	Cellulitis, osteomyelitis	Amphotericin	Abscess, drainage material	1/17/61–1/23/61	1,350	216 (4)	Unchanged	Unchanged

with disseminated disease the period of treatment was too short to evaluate effectiveness (table 6).

Case Reports

Patient 14, A.J., an 82-year-old white housewife with arteriosclerotic heart disease, in 1954 developed bilateral pulmonary coccidioidomycosis with left hydropneumothorax and persistent bronchopleural fistula. She produced 250 to 500 ml. of purulent and sometimes bloody sputum daily, and there was a gradual weight loss of 70 pounds. A coccidioidin skin test was negative and the complement-fixation test was positive in serum dilution of 1:32. Sputum cultures repeatedly yielded *C. immitis*. She had hyperglobulinemia and a high erythrocyte sedimentation rate. The patient failed to respond to a long course of amphotericin by mouth and to 2,500 mg. given intravenously over a period of several weeks. She was given 4.2 gm. of X-5079C in a period of one week only, but no improvement was observed.

Patient 21, J.F., a 25-year-old Negro housewife, had disseminated coccidioidomycosis known since July, 1957, and manifested on admission to the Clinical Center on June 9, 1958, by subcutaneous abscesses of the left shoulder, supraclavicular fossa, groin, paravertebral thoracic area, and thigh; septic arthritis of the right fifth metacarpal phalangeal joint and left knee; osteomyelitis of the thoracic vertebrae 10, 11, and 12, pubis, left fifth proximal phalanx, clavicle, sternum, and tibia; pneumonic infiltration of the right upper lobe and empyema. Numerous cultures of sputum and pus from multiple draining lesions were positive for *C. immitis*. A coccidioidin skin test was negative and a complement-fixation test in serum dilution of 1:128 was positive. The patient did not improve on a short course of X-5079C in June, 1958. Subsequently, there was improvement after many surgical incisions and drainages and a six-month course of amphotericin B intravenously, begun on September 16, 1958, with a total dosage of 13,000 mg. Following discharge on March 27, 1959, clinical improvement continued with healing of the draining lesions; and at readmission in June, 1959, cultures of gastric fluid were negative. Subsequently, the patient became pregnant (last menstrual period late June, 1959). In September, 1959, a subcutaneous mass appeared from which, on return to the Clinical Center in November, 1959, *C. immitis* was again cultured. Other abscesses with draining sinuses subsequently developed in the left ankle, sternum, and left posterior cervical

areas. A second course of X-5079C therapy for six weeks did not exert detectable beneficial effect.

Patient 33, P.G., a 23-year-old Negro male, while in the Air Force and stationed in Arizona in July, 1955, had pulmonary disease diagnosed as coccidioidomycosis from which he seemingly recovered by the time of discharge in September, 1957. In November, 1958, he began to have headaches and, following studies which indicated posterior fossal tumor, underwent craniotomy. A multilobular cystic mass containing pus was removed from the right vermis of the cerebellum. Cultures of operative specimens, cerebrospinal fluid, and sputum were positive for *C. immitis.* The patient improved clinically and cultures became negative during a four-week course of amphotericin intravenously, ending April 15, 1959. Coccidioidin complement-fixation in serum dilution of 1:128 and skin tests were positive. He was admitted to the Clinical Center on February 18, 1959, with an exacerbation of coccidioidal meningitis, and died after twelve days of therapy with X-5079C.

Patient 66, T.M., a 52-year-old white housewife, spent one month in the San Joaquin Valley in 1930, following which she developed a chronic productive cough. Coccidioidomycosis was diagnosed following the isolation of the fungus in 1948, and in 1950 she underwent decortication and thoracoplasty. Two subsequent surgical procedures, including lobectomy, were performed. On admission to the Clinical Center on September 30, 1959, major symptoms were night sweats, fever, chronic productive cough, and dyspnea on exertion. A chest film showed chronic inflammatory changes bilaterally. Tomograms revealed multiloculated cavities and two larger cavities measuring 3 and 6 cm. in the right lung. Hyperglobulinemia, and elevated erythrocyte sedimentation rate (85 mm. per hour), and repeatedly positive sputum cultures for *Coccidioides immitis* were noted. A coccidioidin skin test was negative, and a complement-fixation test was positive in serum dilution of 1:32. Cultures continued to be positive after a six-week course of amphotericin (1,600 mg.). A ten-week course of X-5079C similarly failed to change the cultural status or the chest films.

Patient 93, H. E., a 41-year-old white male automobile salesman developed cough, malaise, fever, night sweats, and chills while in California in June, 1960. A diagnosis of pulmonary coccidioidomycosis was confirmed by positive sputum cultures. In October he was admitted to the Clinical Center where chest films showed a mottled infiltrate involving the entire right lobe and a 13- by 20-mm. cavity on tomograms. A coccidioidin skin test was negative and a complement-fixation test was positive in serum dilution of 1:32. Therapy with X-5079C was associated with cessation of night sweats but no other objective improvement, and sputum cultures remained positive.

Patient 97, H.F., a 32-year-old Negro male laborer, developed disseminated coccidioidomycosis with multiple subcutaneous abscesses and draining sinuses in October, 1952. In 1955, he suffered a traumatic fracture of the right femur that never united and became the site of coccidioidal osteomyelitis. Coccidioidin skin and complement-fixation tests were negative. After treatment with amphotericin and because of this nonunion, amputation of the extremity was performed on January 18, 1961. Immediately before and after this surgical procedure the patient received X-5079C for a period too short to evaluate therapeutic benefit.

Candidiasis

The condition of 2 patients with Candida endocarditis was unchanged and both died despite therapy (table 7). The clinical course of one of these is shown in figure 2.

Case Reports

Patient 45, H.D., a 48-year-old white male dentist, on June 4, 1959, had an aortic valvulotomy for stenosis, from which he made a satisfactory recovery and was discharged on June 20. On approximately July 5, he noted the onset of fever, chilliness, and malaise. On readmission on July 20, no signs of peripheral embolization were present, and heart murmurs were unchanged. Temperature elevations were observed, however. Cultures

TABLE 7

CANDIDA ENDOCARDITIS TREATMENT WITH X-5079C

Number	Patient	Age in Years	Type of Disease	Pevious Treatment	Sites of Positive Cultures	Dates of Treament	Total Dose	Maximal Daily Dose	Cultural Status	Clinical Status
							mg.	*mg.(mg./kg.)*		
45	H.D.	48	*C. guilliermondii* endocarditis	Amphotericin	Blood	9/21/59–11/6/59	15,250	375 (6)	Positive	Dead
68	H.R.	22	*C. parapsilosis* endocarditis	None	Blood, bone marrow	12/26/59–1/22/60	4,440	160 (3)	Positive	Dead

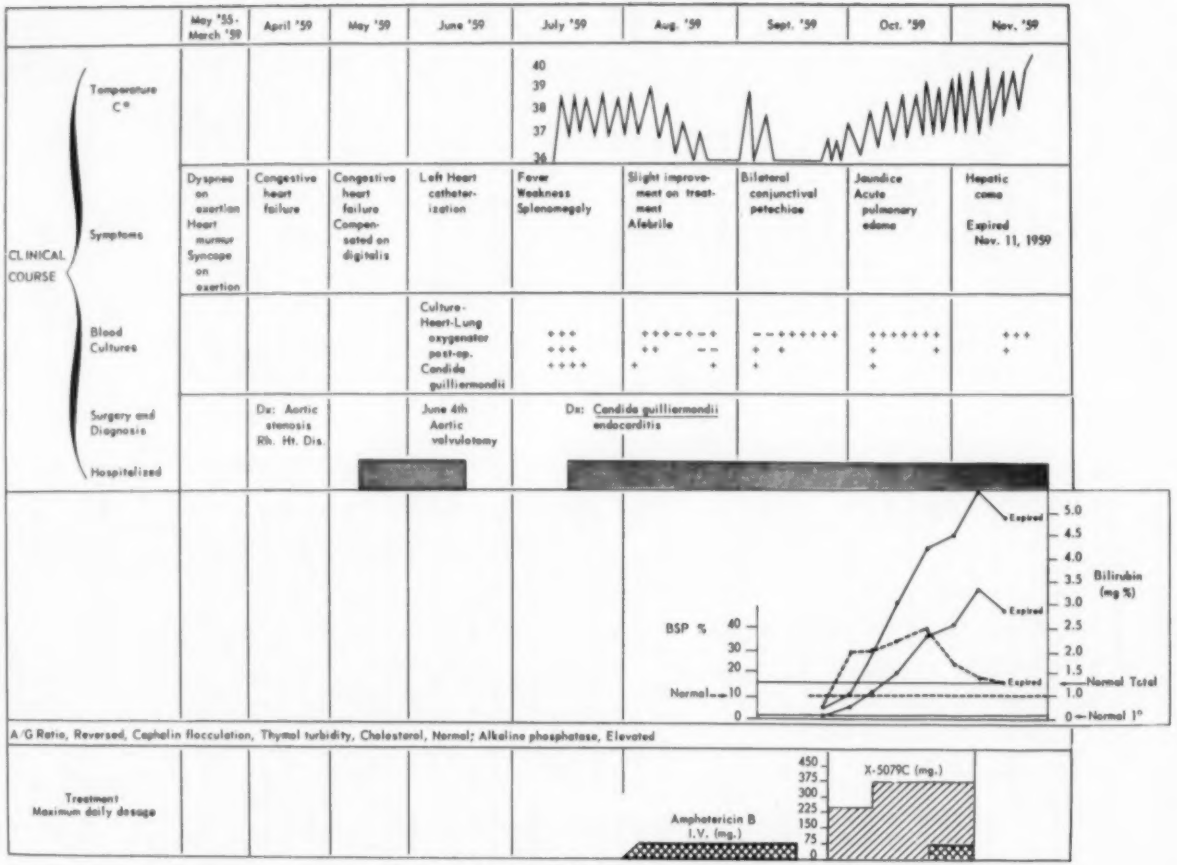


FIG. 2. *Patient 45* with Candida endocarditis.

were positive for Staphylococcus in 2 of 6 media on the first 2 cultures and for *Candida guilliermondii* in 46 of 53 cultures during the subsequent two and one-half months. After institution of vancomycin therapy, bacterial blood cultures became negative. Amphotericin therapy (1,644 mg.) produced no beneficial results. X-5079C was similarly ineffective, and the patient died on November 11, 1959.

Patient 68, H.R., a 22-year-old white male drug addict, took his last intravenous injection of heroin on March 12, 1959, and on the same day was arrested. On June 5, at a routine physical examination in prison, splenomegaly was noted. On June 13, petechiae and fever were observed, and on June 18, 24, and 27, blood cultures were positive for *Candida parapsilosis*. Over the next six months multiple blood cultures and a bone marrow culture were positive. Cardiac murmurs, hematuria, physical findings of embolic phenomena to liver and spleen, and weight loss developed. On admission to the Clinical Center on December 21, the patient appeared wasted and chronically ill with a pansystolic murmur, hepatosplenomegaly, friction rub over the right abdomen, and clubbing of the fingers. The erythrocyte sedimentation rate was 120 mm. per hour. The blood urea nitrogen was 58 mg.; creatinin, 3.0 mg.; globulins, 6.5 gm.; and cholesterol, 82 mg. per 100 ml. Thirty-one additional blood cultures and one more bone marrow culture were positive. Four weeks therapy with X-5079C did not affect clinical course or cultural status, and the patient died.

Discussion

Clinical and cultural data obtained in this study support the diagnosis of severe specific fungal infection in each of the patients described. Specific effective antimicrobial agents for use in these infections have been lacking until the recent use of amphotericin. Even with this drug treatment, failures have occurred (6), and some of the patients included in this series had not recovered on amphotericin therapy. Difficulties in treating these infections are further attested to by fatal outcomes commonly recorded in the past and, indeed, demonstrated in this study.

The serious nature of these infections militates against the general use of placebos or withholding treatment completely for the purpose of obtaining a control series. This study, like others on antifungal therapy in systemic mycoses, is thus not controlled either in single- or in double-blind fashion. The difficulties of drawing conclusions are additionally apparent when the possibility of spontaneous remission in selected cases of blastomycosis or the lack of knowledge of the ultimate prognosis of such recently described forms of disease as chronic cavitary histoplasmosis are considered.

Allowing for these reservations, the drug X-5079C would seem to be an active agent in a variety of fungous infections, including histoplasmosis, blastomycosis, aspergillosis, sporotrichosis, and the one atypical case of maduromycosis. The results of its use in experimental coccidioidomycosis in mice suggest the desirability of additional clinical trials, although the writers' own clinical experience to date is not encouraging. The drug was ineffective in the patients with severe Candida infections.

Summary

Twenty-eight systemic mycotic infections in 27 patients have been treated with X-5079C. The diseases treated include: histoplasmosis (8 cases), blastomycosis (6 cases), coccidioidomycosis (6 cases), aspergillosis (2 cases), sporotrichosis (3 cases), Candida endocarditis (2 cases), and maduromycosis (one case). The drug was administered subcutaneously every six hours, and the maximal daily dosage was usually 4 mg. per kg. (range 3 to 17 mg. per kg.). The duration of therapy was usually four weeks (range one to ten weeks). X-5079C appears to be an active agent in histoplasmosis, blastomycosis, aspergillosis, sporotrichosis, and maduromycosis. No benefit was observed in cases of coccidioidomycosis or Candida endocarditis.

Sumario

Actividad Quimioterapéutica del X-5079C en las Micosis Generalizdas del Hombre

Con el X-5079C se han tratado 28 infecciones micóticas en 27 enfermos. Las enfermedades tratadas comprenden: histoplasmosis (8 casos), blastomicosis (6 casos) coccidioidomicosis (6 casos), aspergilosis (2 casos), esporotricosis (3 casos), endocarditis por Candida (2 casos), y maduromicosis (1 caso). El medicamento fué administrado subcutáneamente cada seis horas y la dosis diaria máxima fué por lo general de 4 mg. por kg. de peso (límites, 3 a 17 mg. por kg.). La duración de la terapéutica fué por lo general de cuatro semanas (límites, una a diez semanas). El X-5079C pareció ser un agente activo en la histoplasmosis, la blastomicosis, la aspergilosis, la esporotricosis y la maduromicosis. No se observó beneficio alguno en casos de coccidioidomicosis o de endocarditis por Candida.

Resume

Activité chimiotherapeutique de X-5079C dans les mycoses systématiques de l'homme

Vingt-huit infections mycotiques systémiques ont été traitées par le X-5079C. Les maladies traitées comprennent: histoplasmose (8 cas), blastomycose (6 cas), coccidioidomycose (6 cas), aspergillose (2 cas), sporotrichose (3 cas), endocardite à Candida (2 cas), et maduromycose (un cas). Le médicament fut administré par voie souscutanée, toutes les 6 heures, et la dose maxima quotidienne fut ordinairement de 4 mg par kg (allant de 3 à 17 mg par kg). La durée du traitement fut ordinairement de quatre semaines (allant de une à dix semaines). X-5079C semble être un médicament actif dans l'histoplasmose, la blastomycose, l'aspergillose, la sporotrichose et la maduromycose. On n'observa aucun bénéfice dans les cas de coccidioidomycose ou d'endocardite à Candida.

Acknowledgment

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EFFECT OF THE ANTIFUNGAL AGENT X-5079C ON THE GROWTH OF HISTOPLASMA CAPSULATUM IN VITRO[1, 2]

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INTRODUCTION

The antifungal agent X-5079C[3] has been shown by Emmons (1) to be effective in modifying the course of experimental histoplasmosis in mice. Early impressions of the low activity of this agent against pathogenic fungi *in vitro* prompted a study of its effect on *Histoplasma capsulatum* in HeLa cell cultures and an examination of the susceptibility of several strains in both the mycelial and yeast forms in broth culture.

MATERIALS AND METHODS

The three strains of *H. capsulatum* used in this study were from the departmental collection and were of human origin. They were maintained in the yeast form by twice-weekly transfers on glucose-cystine–blood agar (2) at 37°C.

Lot No. 43 of X-5079C was used throughout the study except when noted. It was stored in the solid state at 4°C. Solutions were prepared just before use, and sterilized by filtration through an HA millipore membrane.

The susceptibility of each strain of *H. capsulatum* to the antifungal agent was determined in modified Sabouraud broth (2 per cent glucose and 1 per cent neopeptone). Duplicate series of 25-ml. cultures were prepared in 125-ml. flasks containing the drug in serial dilution. The dilution intervals were twofold or less. Care was taken to bracket the concentration which accomplished a 50 per cent reduction in the dry weight of cells produced in five days. The yeast-cell inoculum was taken from seventy-two–hour glucose-cystine–blood agar slants and adjusted to an initial cell density of 10^6 cells per ml. by direct microscopic count. Yeast-form cultures were incubated at 37°C. without agitation. Conversion of the yeast form to mycelium during the five-day incubation period was negligible. Disperse suspensions of mycelium for inoculation were obtained in modified Sabouraud broth by heavily inoculating a stirred flask of medium with yeast cells and incubating the culture for forty-eight hours at 24°C. The inoculum of the mycelial form was adjusted to an initial density of 0.01 mg. per ml., and incubation was at 30°C. without agitation.

Yields of cells were determined by filtering the cultures on HA millipore filters, washing, drying to constant weight at 105°C., and weighing. The concentration of the antifungal agent which caused a 50 per cent reduction in yield of cell mass was determined graphically by the use of a normal curve-deviate scale for representation of the per cent of growth response and a logarithmic scale for the concentration of drug (3).

The cultivation of *H. capsulatum* in HeLa cell culture has been described by Larsh and Shepard (4), and the procedure used in this study was similar to that method. Leighton tubes were inoculated with 3×10^5 HeLa cells per tube in 1 ml. of Eagle medium containing 10 per cent bovine serum, 100 units of penicillin, and 100 γ of streptomycin. The cultures were incubated for forty-eight hours at 37°C. with rubber stoppers to maintain a suitable carbon dioxide concentration. The medium was then replaced with 1 ml. of Eagle medium containing 20 per cent fresh guinea pig serum and 10^4 cells of yeast-form *H. capsulatum* from seventy-two–hour glucose-cystine–blood agar slants. After incubation for twenty-four hours at 37°C., the tubes were opened and X-5079C in 0.05 ml. of Eagle medium was added. The cultures were then incubated for an additional five days at either 37° or 30°C., as indicated. At forty-eight hours after inoculation, and again at ninety-six hours, the growth medium was replaced with Eagle medium containing 10 per cent bovine serum and the appropriate amount of the antifungal agent.

Tissue-cell cultures which were intended for microscopic examination were prepared in Leighton tubes containing a 9- × 22-mm. coverglass. The HeLa cell cultures were washed with saline, fixed in absolute methanol, and stained in the tubes by the periodic acid–Schiff method (5). The coverglasses were then removed, mounted, and examined.

Replicate cultures intended for subculture on agar slants were grown in Leighton tubes without coverglasses. At the end of the growth period, the cultures were washed twice with Eagle medium, treated for seven or eight minutes with 0.2 ml. of 0.25 per cent trypsin, diluted to 2.0 ml. with Eagle

[1] From the U. S. Department of Health, Education, and Welfare, Public Health Service, National Institute of Allergy and Infectious Diseases, Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 22, 1961.

[3] The X-5079C used in this study was furnished by Hoffmann-La Roche, Inc., Nutley, New Jersey.

medium, and dispersed with a pipette and rubber bulb. Serial tenfold dilutions in Eagle medium were made to ensure that subculture of 0.2-ml. quantities on agar slants produced discrete colonies that could be counted. Strains 6641 and 6650 were subcultured on glucose-cystine–blood agar slants. Modified Sabouraud agar was used for strain 6640 since there were indications that this medium produced higher counts with this strain than the glucose-cystine–blood agar medium. The agar slants were incubated for ten days at 30°C. before colony counts were made.

Results

Fungicidal activity of X-5079C: Yeast-form cells of strain 6640 of *H. capsulatum* were suspended in modified Sabouraud broth at a density of 5×10^5 particles per ml. The majority of particles consisted of one and two cells with a few larger clusters. Parallel suspensions were incubated at 37°C. for six hours with and without 100 γ of X-5079C per ml. Samples were withdrawn at intervals of two hours, serial dilutions were made, and 0.2-ml. volumes were spread on modified Sabouraud agar plates. Colony counts were made after incubation at 30°C. for ten days. There was no evidence of a decrease in the number of colony-forming particles in the presence of the agent as compared with the control suspension. It was concluded that under the conditions here employed, X-5079C was not fungicidal for *H. capsulatum*.

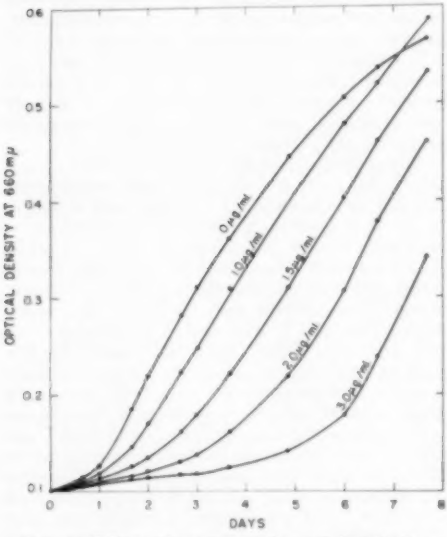

FIG. 1. Effect of concentration of X-5079C on the growth of the yeast form of *Histoplasma capsulatum*.

TABLE 1

SUSCEPTIBILITY OF THE YEAST AND MYCELIAL FORMS OF HISTOPLASMA CAPSULATUM TO THE ANTIFUNGAL AGENT X-5079C

Strain Number	X-5079C Producing 50 Per Cent Inhibition of Growth γ per ml.	
	Yeast Form	Mycelial Form
6640	2.15	6.7
6641	1.40	8.4
6650	1.32	23.3

Effect of X-5079C on the growth of H. capsulatum *in modified Sabouraud broth:* The yeast form of strain 6650, unlike the other two strains studied, was capable of disperse growth in modified Sabouraud broth. The growth of this strain could therefore be measured by optical methods. Cultures were prepared in optically standardized 20- × 150-mm. culture tubes closed with stainless steel caps. Each tube received 6.0 ml. of modified Sabouraud broth containing 10^6 *H. capsulatum* cells per ml. and the indicated concentration of the antifungal agent. Five tubes were prepared at each concentration. The cultures were incubated at 37°C. and shaken only at the time of measurement. Optical density determinations were made at intervals at 660 mμ with a model B Beckman spectrophotometer. The results are summarized in figure 1. It is apparent that the effect of the antifungal agent is to delay growth which ultimately proceeds at a normal rate. Such a delay in growth may be caused by a change in the susceptibility of the population of yeast cells, a decrease in the activity of the agent, or both.

In order to test the stability of the drug in modified Sabouraud medium, a solution containing 12 γ per ml. was incubated at 30°C. for eight days. Strain 6650 of *H. capsulatum* was used to determine the antifungal activity of the solution by the optical density method, with freshly prepared X-5079C solutions as standards. It was found that 25 per cent of the activity was lost in two days and 82 per cent in eight days.

In table 1 the results are summarized of susceptibility tests of the yeast and mycelial forms of the three strains of *H. capsulatum*, expressed as concentration of agent required to cause a 50 per cent reduction in the cell mass

produced in five days. Each value reported is the average of two or more determinations.

Activity of X-5079C in HeLa cell culture: The antifungal agent had no obvious effect on the growth and appearance of HeLa cells when present in the culture fluid at a concentration of 100γ per ml. Moreover, there was no apparent effect of 10γ of the drug per ml. on the phagocytosis of formalin-killed *H. capsulatum* by HeLa cells. Microscopic examination of stained preparations of HeLa cell cultures inoculated with *H. capsulatum* indicated a suppression of growth of the fungus in the presence of 1 to 5γ of X-5079C per ml. The results of the microscopic examinations are not reported here in detail because of the difficulty of quantitating the amount of *H. capsulatum* appearing in the stained preparations and the inability to distinguish between dead and viable fungous cells. However, the appearance of the slides confirmed the results of the subcultures, which are summarized in table 2. It is apparent that concentrations of X-5079C which inhibited growth of the yeast form of *H. capsulatum* in broth culture are also effective in HeLa cell culture. It is also noteworthy that growth of the fungus was greatly enhanced by incubation at 30°C., as compared with 37°C. At the lower temperature there was some development of mycelium, varying with the strain, but it is probable that the mycelium contributed in only a minor way to the colony count because of a greater resistance to dispersion of the mycelial form of the fungus. At higher concentrations of the antifungal agent, the mycelial growth may contribute a greater proportion to the total count because of the very limited development or persistence of yeast cells. At 37°C., growth of the fungus was limited, perhaps by competition with the HeLa cells for oxygen in the sealed tubes, as well as by an inherently lower growth rate at 37°C. Some improvement in growth was noted when the tubes were plugged with cotton and placed in a 37°C.-incubator with an atmosphere controlled at 4 per cent carbon dioxide and 96 per cent air.

Discussion

The results of the determinations of the susceptibility of three strains of *H. capsulatum* to the antifungal agent X-5079C indicate a significant difference between the yeast and mycelial forms. Since the tests with the two forms of the fungus were carried out at different temperatures, it would be hazardous to attribute the differences in susceptibility solely to the difference in morphology. However, the similar susceptibility of the fungus at 30°C. and 37°C. in HeLa cell culture suggests that temperature does not play a major role and that the differences noted are directly related to morphology. Recently (6) a similar relationship has been reported between the susceptibility to cycloheximide of the two forms of the dimorphic fungi, *Blastomyces dermatiditis, Histoplasma capsulatum, Paracoccidioides brasiliensis,* and *Sporotrichum schenkii.*

Because the purity of Lot No. 43 of X-5079C used in these studies is not known, the susceptibility values reported cannot be taken as absolute. A comparison of Lot No. 43 with an earlier lot, No. 29, indicated an activity of 68 to 70 per cent of the latter. It is apparent from this

TABLE 2

Effect of X-5079C on Three Strains of Histoplasma Capsulatum in HeLa Cell Culture

X-5079C	Strain 6640				Strain 6641				Strain 6650			
	37°C.		30°C.		37°C.		30°C.		37°C.		30°C.	
	C.F.P.* per Tube	Per Cent of Control	C.F.P. per Tube	Per Cent of Control	C.F.P. per Tube	Per Cent of Control	C.F.P. per Tube	Per Cent of Control	C.F.P. per Tube	Per Cent of Control	C.F.P. per Tube	Per Cent of Control
γ per ml.												
0	2,050	100.0	70,000	100.0	2,400	100.0	102,000	100.0	2,200	100.0	160,000	100.0
1	1,750	85.0	80,000	114.0	195	8.1	20,500	20.0	50	2.3	14,000	8.8
2	205	10.0	1,950	2.8	110	4.6	1,400	1.4	20	0.9	550	0.3
3	215	11.0	950	1.4	75	3.1	1,400	1.4	25	1.1	100	0.06
5	65	3.2	700	1.0	40	1.7	900	0.9	<10	<0.5	100	0.06

* C.F.P. = Colony-forming particles

and the demonstrated instability of the agent in the growth medium that the strains of *H. capsulatum* tested are more susceptible to the antifungal agent than the tabulations indicate. Emmons and Piggott (7) have reported serum concentrations of X-5079C of 2 to 11γ per ml. in patients receiving treatment with this drug, a range of concentration adequate to inhibit the growth of the yeast form of *H. capsulatum* in broth and in HeLa cell culture.

The delayed growth of *H. capsulatum* in cultures containing the antifungal agent can be explained by the demonstrated instability of the agent, although the possibility of outgrowth of more resistant forms has not yet been ruled out. The results emphasize the need for restricting the time of incubation in susceptibility testing to as short a period as is consistent with a reliable measurement of growth. The use of the mycelial growth form and long incubation periods will give the impression that *H. capsulatum* is relatively resistant to inhibition by the new antifungal agent.

Summary

The susceptibility of three strains of *Histoplasma capsulatum* to the antifungal agent X-5079C has been determined. The substance was found to be fungistatic rather than fungicidal for this microorganism. The agent lost activity gradually in Sabouraud broth and, consequently, delayed growth of *H. capsulatum* occurred in cultures containing the drug. Growth of the yeast form of *H. capsulatum* at 37°C. was inhibited by concentrations of the antifungal agent which have been reported present in the serum of patients undergoing treatment with the drug. The mycelial form of the fungus at 30°C. was more resistant to X-5079C than the yeast form.

At a concentration of 100γ per ml., the substance was not toxic for HeLa cells in culture. The susceptibility of *H. capsulatum* yeast cells growing in HeLa cell culture was comparable to that in Saburaud broth.

Sumario

Efecto del Agente Antifungoso X-5079C sobre la Proliferación del Histoplasma Capsulatum in Vitro

Se ha determinado la susceptibilidad de tres cepas del *Histoplasma capsulatum* al agente antifungoso X-5079C. La sustancia resultó ser fungistática más bien que fungicida para dicho microorganismo. El X-5079C perdió gradualmente su actividad en el caldo de Sabouraud y, por consiguiente, hubo proliferación retardada del *H. capsulatum* en cultivos que contenían la droga. La proliferación de la forma en levadura del *H. capsulatum* a 37°C. fué inhibida por concentraciones del agente antifungoso que, según se ha comunicado, existían en el suero de enfermos sometidos a tratamiento con la droga. La forma micelial del hongo, a 37°C., fué más resistente al X-5079C que la forma en levadura.

A una concentración de 100 γ por ml., la sustancia no resultó tóxica para las células de HeLa en cultivo. La susceptibilidad de las células en levadura del *H. capsulatum* en proliferación en cultivo de células HeLa fué comparable a la observada en el caldo de Sabouraud.

Resume

Effet du médicament antifungique X-5079C sur le développement d'Histoplasma capsulatum in vitro

Les auteurs ont determiné la sensibilité de trois souches *d'Histoplasma capsulatum* vis à vis du médicament antifungique X-5079C. On a trouvé que le produit était plus fungistatique que fungicide vis à vis de cet organisme. X-5079C perdit petit à petit son activité dans le bouillon de Sabouraud, et, en conséquence, un développement retardé de *H. capsulatum* se produisit dans les cultures contenant le médicament. Le développement de la forme "levure" de *H. capsulatum* à 37°C fut inhibé par les concentrations du médicament antifungique qui ont été trouvées présentes dans le sérum des malades subissant le traitement par ce médicament. La forme mycéliale du champignon à 30°C fut plus résistante vis à vis de X-5079C que la forme "levure."

A la concentration de 100 γ par ml, la substance ne fut pas toxique pour les cellules de HelA en cultures. La sensibilité des cellules "levure" de *H. capsulatum* poussant dans une culture de cellules de HelA fut comparable à celle du bouillon de Sabouraud.

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BIOASSAY OF THE ANTIFUNGAL AGENT X-5079C IN SERUM OF PATIENTS[1, 2]

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INTRODUCTION

Clinical trials of a new drug should include assay of the drug in the patient's serum. An accurate assay may indicate whether the drug has reached a presumably effective concentration in the serum, whether increases in dosage achieve higher serum concentrations, and whether signs or symptoms of toxicity are associated with serum concentrations beyond a therapeutic concentration.

In experimental tests and clinical trials of the new antifungal agent X-5079C[3] (1–4), chemical methods of assay in serum are not available and methods of bioassay present unusual problems. The agent is fungistatic rather than fungicidal and decays rapidly (5), so that Histoplasma grows in broth containing 65 mg. of the drug in each milliliter of the solution. However, initial growth is limited in amount, perhaps only to that permitted by endogenous nutrients. Renewed growth occurs after decay of the antifungal agent (5). On the other hand, there is *in vivo* activity at low concentrations. Mice are protected from death by intraperitoneal administration of X-5079C for only a few days at the dosage level of 5.3 mg. per kg., although they will tolerate daily intraperitoneal injections at more than fifty times that level (2). In clinical trials (3) dosage has usually been kept below 5 mg. per kg. Demonstrable serum concentrations remain low and cannot be measured conveniently by the usual bioassay methods of serum dilution in broth because of the fungistatic activity, the decay of the drug, and the large volume of serum required.

Assay for the drug after it has been incubated with sterile fresh mouse tissue has not demonstrated its conversion in this type of *in vitro* test to a more active form.

METHODS

Berger (1), after screening X-5079C against many fungi, selected a strain of *Paecilomyces varioti* which is suitable for bioassay of the drug. Even this fungus, in broth-dilution tubes, does not conveniently detect serum concentrations achieved in either experimental or clinical therapeutic trials. However, in a seeded agar culture, although spores of the fungus germinate, growth of aerial mycelium is restricted and sporulation is inhibited in zones which surround filter paper discs impregnated with the agent or with serum containing it.

The bioassay of X-5079C is based upon a comparison of the widths of zones of inhibition around two sets of discs, one set having been soaked in the sera to be tested and the other in graded dilutons of the drug in 0.85 per cent sodium chloride solution. Accuracy of the test is based upon the fortunate circumstance that a nearly straight-line relationship exists between the logarithm of the drug concentration and the widths of zones of inhibition within the range of serum concentrations reached in therapeutic use.

The following procedures give uniform and reproducible bioassay. Discs ¼-inch in diameter are punched from separated sheets of Whatman #3 filter paper. If punched from a stack of sheets, separation of discs is difficult in later handling. The discs are sterilized in lots of six in 13- × 100-mm. test tubes for convenience in making tests in replicates of six.

A suspension of spores of *P. varioti* is prepared from cultures on three slants (16- × 150-mm. culture tubes) of Sabouraud agar seven to fourteen days old by adding 7 ml. of 0.85 per cent sodium chloride containing 0.05 per cent Tween® 80 to each of two slants. The fluid is carefully pipetted down the side of the tube opposite the slant, the spores are rubbed loose with a bacteriologic loop, and the spore suspension is transferred by pipette to a test tube and made up to a volume of 15 ml. with Tween-saline. The spore chains are dissociated by being forcibly drawn into and forced out of a pipette and allowed to stand for five minutes. Ten milliliters of spore suspension are withdrawn from the central portion of the liquid column, added to 150 ml. of melted horse-meat infusion agar cooled to 40°C., and poured into flat-bottomed Petri plates to a depth not to exceed 2 to 3 mm. After the agar

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[2] Presented in part before the Medical Session, as part of *Section 2B*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 22, 1961.

[3] The X-5079C used in this study was furnished by Hoffmann-La Roche, Inc., Nutley, New Jersey.

has hardened, the covers are replaced with other sterile dry covers and the plates are left to dry at room temperature for four to six hours.

A standard solution is prepared by dissolving 10 to 20 mg. of X-5079C in 0.85 per cent sodium chloride, and making this up to 1,350 γ per ml. This is diluted 1:100. From this standard solution, logarithmic dilutions are selected to cover short intervals within the range of antifungal agent expected, or determined by preliminary test, to be in the serum. Twofold dilutions provide too few points within this range. Serial 2:3 dilutions are made in this laboratory by adding 10 ml. of drug solution to 5 ml. of saline, giving the series, 13.5, 9.0, 6.0, 4.0, and 2.7 γ per ml. When the series is reversed for plotting purposes, the concentration of the drug in each dilution is 1.5 times that of the preceding dilution.

One milliliter of each dilution of the drug is added to a tube containing the previously sterilized paper discs. Similarly, each undiluted serum specimen to be tested is added to a tube containing six discs.

One disc is removed from one of the standard drug dilutions with a flamed loop, it is touched several times to the inner side of the tube in order to drain off excess solution, and placed carefully without lateral sliding on the seeded agar surface. Similarly, discs are removed from the other three dilutions and from as many serum specimens (up to five) as are to be tested simultaneously. Six replicate plates are prepared in this way. To increase precision of the test, the discs are placed equidistantly from the center of the plate and spaced uniformly from each other. Eight discs per plate allow sufficient distance between zones of inhibition.

It is difficult to remove discs with a loop made of heavy wire, and a long loop of fine wire is too flexible. For convenience, therefore, a loop of 22-gauge nicochrome wire is made and lashed to the end of a straight piece of 18-gauge nicochrome wire, thus giving a stiff wire with a loop of fine wire at the end.

Recent experience indicates that zones are sharper if incubation is at room temperature rather than at 37°C. However, most of the assays in this laboratory have been made as follows. After incubation of the plates at 37°C. for sixteen hours, the plates are removed to room temperature for four hours. Replicate measurements are made of each zone of inhibition (total diameter of zone and disc) with calipers, and the readings of the six replicate tests of each dilution of the standard solution, as well as each serum specimen, are averaged. No appreciable changes in diameters of zones occur during the interval between sixteen and twenty hours, although the limits of the zones become sharper. The zones remain readable for several days. The concentration of antifungal agent is estimated in serum samples by comparison of diameters of zones of inhibition with those of standard dilutions.

Results

Diameters of zones in replicate testing of a serum specimen or standard dilution, tests of many serum specimens from patients receiving X-5079C therapy, and retesting of serum after freezing have demonstrated that this quantitative bioassay is reasonably accurate (table 1 and figure 1). The lowest concentration of the X-5079C standard dilution (2.7 γ per ml.) produces barely measurable zones of inhibition. On occasional plates it gives no detectable zones and it is, therefore, near the lower limit of detectable concentrations. At the other extreme, this laboratory has tested no serum with a concentration greater than 11.0 γ per ml. This is lower than the highest concentration of the standard (13.5 γ per ml.) used in assay. The serum of patients under treatment contained detectable concentrations of the agent only after two or three days

TABLE 1

Bioassay for X-5079C. Serum of Patient on Usual Regimen just Prior to and Following Subcutaneous Injection of 1 Mg. per Kg.

Plate Number	Zone Diameter in Millimeters							
	Standards, Mg. per Ml.				Serum at Indicated Hours			
	0.0025	0.005	0.010	0.020	Zero	1	3	5
1	8	11.5	16.0	18.5	13.0	12.5	12.5	12.5
2	7	11.5	15.5	19.0	11.5	12.0	11.0	12.0
3	7	11.5	15.0	18.0	11.0	11.5	12.0	11.0
4	9	14.0	16.0	18.0	12.0	11.0	12.0	12.0
5	8	11.0	16.0	18.5	13.0	12.0	12.0	12.0
6	8	12.0	16.0	19.0	13.0	12.0	12.0	11.5
Total	47.0	71.5	94.5	110.0	73.5	71.0	71.5	71.0
Average	7.83	11.92	15.75	18.33	12.25	11.83	11.92	11.83

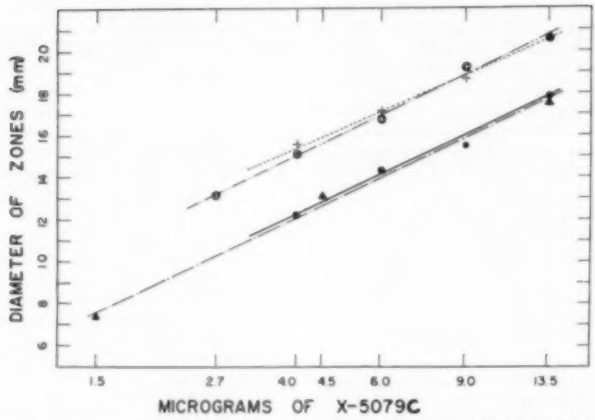

FIG. 1. Diameters of zones of inhibition produced by dilutions of X-5079C in four experiments.

of treatment, and continued therapy did not raise the concentration above that reached during the first week of therapy. Detectable serum concentrations remained for one or two days after therapy was stopped.

DISCUSSION

Within the range of standard dilutions of X-5079C used (2.7 to 13.5 γ per ml.), a good degree of linearity exists between dilution and diameter of zone (figure 1). Fortunately for the accuracy of the test, these values bracket the lowest detectable and the highest observed concentrations of the drug in the serum of patients. This series of dilutions reveals no break in linearity at either barely detectable concentrations or at the highest concentrations of drug found in the serum of treated patients. The errors associated with measurements of zones of inhibition can be reduced by averaging three to five independent measurements of each zone. Errors associated with uneven depth of agar and resultant differences in diameters of zones are reduced by using six replicate plates for each serum assay.

Other critical points which influence the accuracy of the test are the use of horse-meat infusion agar, a uniform depth of seeded agar not in excess of 2 to 3 mm., placement of the discs four to six hours after the seeded agar is poured, and measurement of the zones at sixteen to twenty hours. Germination of spores of *P. varioti* begins after seven hours of incubation at 37°C., and after nine hours' incubation at room temperature. Therefore, on the schedule described, germination has not begun at the time discs are placed on the seeded agar plates.

X-5079C produces narrow and indistinct zones of inhibition if *H. capsulatum* is substituted for *P. varioti* in the bioassay method described. The substitution of Sabouraud agar for horse-meat infusion agar induces better growth of *H. capsulatum*, but the zones of inhibition are less distinct.

SUMMARY

Bioassay of X-5079C by serum dilutions was unsuccessful because it requires large volumes of serum and because of the fungistatic action and decay of the antifungal agent in solution.

By adaptation of the technique of placing impregnated discs on seeded agar, quantitative assays for the drug in serum are possible. The lower detectable limits of X-5079C are near 2.0 γ per ml. of standard solution. This concentration is also detectable in serum. Serum concentrations above 11.0 γ per ml. have not been found in patients receiving the drug.

SUMARIO

Biovaloración del Agente Antifungoso X-5079C en el Suero de los Enfermos

La biovaloración del X-5079C en diluciones del suero sanguíneo no dió resultado debido a que requiere grandes volúmenes de suero y debido a la acción fungistática y al deterioro del agente antifungoso en solución.

Con la adaptación de la técnica de colocar discos impregnados sobre agar sembrado, resulta posible hacer valoraciones cuantitativas para la droga en el suero. Los límites más bajos descubribles para el X-5079C en el suero se aproximan a 2.0 γ por ml. de solución tipo. Esta concentración es también descubrible en el suero. No se han descubierto concentraciones séricas superiores a 11.0 γ por ml. en los enfermos que recibieron la droga.

Resume

Bioréaction au médicament antifungique X-5079C dans le sérum des malades

Une bioréaction de X-5079C avec des dilutions de sérum échoua parcequ'elle nécessite des volumes de sérum importants et à cause de l'action fungistatique et la dégenérescence du médicament antifungique en solution.

En adaptant la technique de placement des disques imprégnés sur l'agar-agar ensemencé, des réactions quantitatives du médicament dans le sérum furent possibles. Les limites les plus basses de X-5079C que l'on put déceler sont proches de 2,0 γ par ml dans une solution standard. Cette concentration est aussi décelable dans le sérum. On n'a pas trouvé de concentrations sériques au dessus de 11,0 γ par ml. chez les malades prenant le médicament.

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ALTERED SULFOBROMOPHTHALEIN METABOLISM IN MAN INDUCED BY THE ANTIFUNGAL AGENT X-5079C[1, 2]

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Introduction

During an evaluation of the therapeutic application in man of a new antifungal agent, X-5079C (1), previously found to be successful in the treatment of experimental mycoses in mice (2, 3), an hepatotoxic effect was observed, apparently reversible and mild in degree. This drug-induced hepatic abnormality was most strikingly and consistently manifested by marked alterations in sulfobromophthalein (BSP) retention values and, to a lesser degree, by abnormalities in other liver function tests and slight alterations in liver morphology. This report describes the pronounced alteration in sulfobromophthalein metabolism and other toxic manifestations of this agent in 28 patients.

Materials and Methods

The antifungal agent, X-5079C, was administered subcutaneously to 28 patients with systemic mycoses in four equally divided doses ranging from 3 to 17 mg. per kg. of body weight per day. Although the exact formula of X-5079C has not yet been determined, the drug is known to be a polypeptide and soluble in water.

Serial laboratory determinations evaluating renal, hematologic, cardiac, and liver function were performed before, during, and—when possible—after treatment. Specifically, studies included routine urinalyses and determinations of blood urea nitrogen, uric acid, creatinine, fasting sugar, sulfobromophthalein, alkaline phosphatase, bilirubin—conjugated and total—total protein, albumin and globulin, thymol turbidity and cephalin flocculation, glutamic-oxaloacetic transaminase, sodium, potassium, bicarbonate, chloride, calcium, phosphorus, amylase, prothrombin time, hematocrit, hemoglobin, erythrocyte sedimentation rate; leukocyte, differential, eosinophil, platelet, and reticulocyte counts. All studies were performed at weekly intervals, or more often when indicated.

In addition, 25 liver biopsies were performed on 11 patients: 6 before, 10 during, and 9, 3 to 307 days after therapy. Autopsy liver specimens obtained on one patient during therapy, and from 4 patients 3, 10, 11, and 25 days after therapy were also studied.

The per cent of sulfobromophthalein retention was determined in all patients by injecting sodium phenoltetrabromophthalein disulfonate (BSP, Hynson, Westcott and Dunning) intravenously in a dose of 5 mg. per kg., and obtaining a blood sample 45 minutes later. Per cent BSP retention was then determined by the method of Gaebler (4). In addition, tests for BSP were performed on X-5079C[4] in human serum with and without BSP added *in vitro*, and the values were identical with the known concentration of the added BSP.

Results

The only significant toxicity was manifested by consistent alterations in tests of liver function.

Effect on sulfobromophthalein: The 45-minute sulfobromophthalein determination was the most markedly altered test of liver function observed in patients receiving the antifungal agent (table 1). In every patient, BSP retention was increased at the time of the first determination and was abnormal in each of 4 patients as early as 5 hours after the administration of the first dose of drug (figure 1). Values promptly reached a plateau (range 16–67 per cent) where they remained except for minor variations throughout therapy (figure 2). When the antifungal agent was discontinued, the BSP value fell rapidly to pretreatment levels within one to 9 days except for *Patient 39*. Four of 5 patients (*Patients 11, 65, 88,* and *99*) with abnormal BSP values before treatment had normal BSP values after treatment; in the remaining patient (*Patient 66*), the BSP value returned to the pretreatment level.

Effect on other liver function tests: Conju-

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[4] The X-5079C used in this study was furnished by Hoffmann-LaRoche, Nutley, New Jersey.

TABLE 1

LIVER FUNCTION IN 28 PATIENTS DURING ADMINISTRATION OF ANTIFUNGAL AGENT X-5079C

Liver Function Test	Number of Patients: in whom test was evaluated	Number of Patients: with rise in test value during therapy	Number of Patients: with return of test value to normal after therapy	Range of Abnormal Values	Per Cent of Patients with Abnormal Values Presumptively Related to Therapy
Sulfobromophthalein	24	24	20*†‡	16–67% retention	100
Conjugated bilirubin	25	22	19*‡	0.32–3.30 mg./100 ml.	88
Unconjugated bilirubin	26	11	8*‡	0.91–2.20 mg./100 ml.	43
Serum glutamic oxaloacetic transaminase	22	4§	3‡	33–62 units 34–148‡ units	9
Alkaline phosphatase	23	6‖	5‡	14–22 K.A.¶ units 32–68‡ K.A. units	9

* Two patients died during therapy.
† One patient died shortly after therapy.
‡ One patient with known cirrhosis and abnormal values, before and after therapy.
§ Two patients with elevated glutamic oxaloacetic transaminase had acute myocardial infarctions.
‖ Three patients had known osteomyelitis and one patient had histoplasmosis of the liver.
¶ King Armstrong.

gated serum bilirubin values rose slightly to abnormal levels (range 0.32 to 3.30 and median 0.95 mg. per 100 ml.) in 22 of 25 patients (table 1). In 18 of the 22 patients this value was abnormal at the time of the first determination during the first week of treatment. These values returned to normal within 3 to 7 days after therapy was stopped (except for *Patient 39*).

Unconjugated serum bilirubin values became abnormal (range 0.91 to 2.20 and median 1.30 mg. per 100 ml.) during therapy in 11 of 26 patients (table 1). In 8 of the 11 patients, abnormal values did not occur until the third week of therapy. Values returned to normal within 2 to 4 days after therapy was discontinued (except for *Patient 39*).

The glutamic oxaloacetic transaminase values became abnormal during therapy in 4 of 22 patients, 2 of whom (*Patients 31* and *68*) had acute myocardial infarctions at the time of the rise; one (*Patient 39*) had cirrhosis and abnormal pretreatment values, and one (*Patient 66*) had coccidioidomycosis and transiently abnormal values which returned to normal 7 days after therapy was completed.

The serum alkaline phosphatase value rose

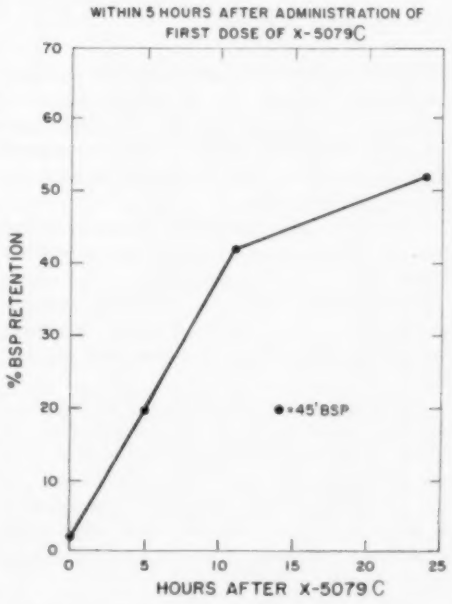

FIG. 1. Rapid rise in sulfobromophthalein retention after first dose of X-5079C.

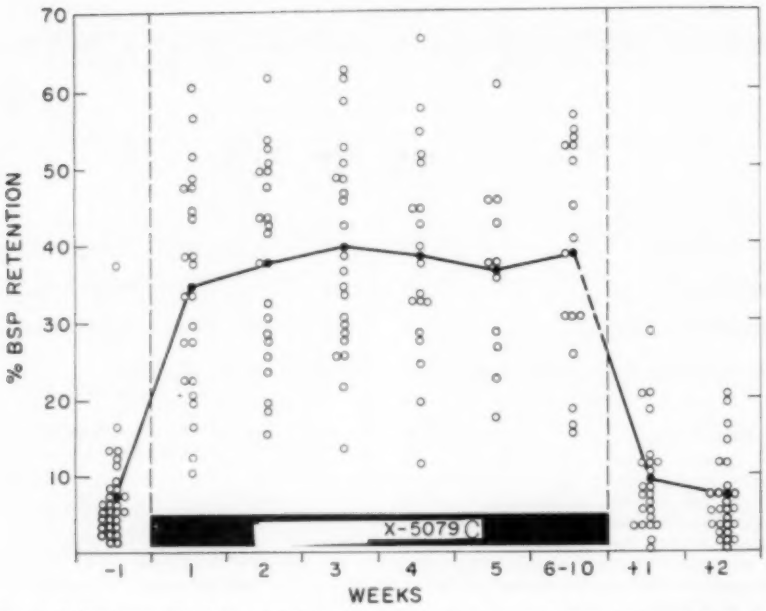

FIG. 2. Sulfobromophthalein retention observed in 28 patients before (−1 week), during, and after (+1, +2 weeks) treatment with antifungal agent X-5079C. Mean values are represented by solid circles.

during therapy to abnormal levels in 6 patients, 3 of whom (*Patients 21, 46,* and *65*) had mycotic osteomyelitis and one (*Patient 88*), histoplasmosis of the liver. Values returned to normal soon after therapy was completed (except for *Patient 39*) (*see* table 1).

Serum glutamic oxaloacetic transaminase and alkaline phosphatase values are not considered significantly altered by X-5079C.

Total protein, albumin, globulin, cephalin flocculation, thymol turbidity, and cholesterol values did not become abnormal and are not listed.

Other effects: Icterus developed during therapy in 4 (*Patients 27, 39, 45,* and *94*) of 28 patients. Two (*Patients 11* and *14*) had transitory pruritis, erythema, and induration at the site of a few injections. One (*Patient 47*) experienced a generalized giant urticaria with eosinophilia which recurred when he received the drug again at a later date.

Changes in liver morphology: Microscopic studies of liver sections of 14 patients were obtained before, at various times during, and after, therapy with X-5079C by means of liver biopsy in 9, biopsy and autopsy in 2, and at autopsy only in 3 patients. In all, 25 liver biopsies and 5 autopsy specimens were examined histologically (table 2).

Before treatment, 3 of 6 biopsies were normal, but biopsies of 2 patients with elevated sulfobromophthalein levels and histories of excessive ethanol ingestion revealed severe chronic inflammation with increased connective tissue in the portal areas (*Patient 66*) and moderate fatty infiltration (*Patient 87*). Biopsy of the remaining patient (*Patient 11*) with symptomatic cholelithiasis and abnormal BSP levels revealed mild chronic inflammation in the portal areas and focal fatty metamorphosis.

During treatment, 9 of 10 liver biopsies obtained from 8 patients at times varying from the seventh to seventieth days of therapy and doses varying from 2.6 to 18.47 gm. of the antifungal agent revealed either the development of, or an increase in, previously existing nonspecific chronic periportal inflammation (figures 3, 4, and 5). Focal granulomatous formation (*Patient 27*), acute focal hepatic necrosis (*Patient 31*), and focal fatty metamorphosis (*Patient 65*) were

observed in addition to the previously mentioned chronic periportal inflammation. Liver biopsy of *Patient 11* obtained on the twenty-eighth day and after 5.4 gm. of the drug was morphologically unchanged from the pretreatment biopsy specimen. An autopsy specimen of *Patient 33* who died on day 13 of therapy after receiving 4.5 gm. of X-5079C revealed moderate centrolobular congestion secondary to congestive heart failure. In all patients, BSP (range 20–67 per cent) and conjugated bilirubin (range 0.28–1.8 mg. per 100 ml.) values were abnormal at the time of liver biopsy. In addition, in many biopsies, subtle evidence of hepatic cell damage, such as changes in cytoplasmic and nuclear staining and variations of nuclear size, was seen.

After treatment, 6 of 9 liver biopsies obtained 37 to 307 days after 2.1 to 28.74 gm. of X-5079C revealed none of the previous nonspecific chronic inflammation in the portal areas, changes in cytoplasmic and nuclear staining, and variations in nuclear size (figure 6). One biopsy (*Patient 88*), obtained 3 days after a total dose of 22.84 gm. of the antifungal agent revealed focal granulomatous inflammation, but no organisms were seen. One biopsy (*Patient 65*) obtained 100 days after a total dose of 10.06 gm. of the drug revealed increased inflammation in the portal areas, small bile duct proliferation, and occasional binucleate hepatic cells. A later biopsy from this patient 307 days after therapy was essentially normal. A biopsy (*Patient 83*) obtained 115 days after a total dose of 11.05 gm. of the antifungal agent revealed focal chronic inflammation of the portal areas (a pretreatment biopsy had not been obtained). Autopsy liver specimens of 4 patients (*Patients 27, 31, 45, 68*) obtained 3 to 25 days following total doses of 3.7 to 15.25 gm. of X-5079C also revealed nonspecific chronic inflammation in the portal areas.

No abnormalities of hematologic, renal, or cardiac function, serum electrolyte, or amylase values were observed.

Discussion

In an attempt to understand the alterations in liver function during the administration of the antifungal agent, the following possible mechanisms must be considered. First, the rapid rise in sulfobromophthalein retention observed in all patients following the administration of the first dose of this drug suggests that it may act in competition with BSP for reduced glutathione in the liver, thereby interfering in the glutathione conjugation of BSP. Although the possible role of conjugation with glutathione in affecting the rate and extent of hepatic uptake, storage, or biliary excretion of BSP has not been defined, halogenated organic compounds other than BSP have been observed to react with reduced glutathione in a similar way, forming thioether derivatives (5). Whether these similar reactions occur non-enzymatically or by enzyme participation of a dehalogenase type has not been established (5–7). Certain choleretic agents have also been shown to delay BSP excretion (8). This mechanism of interference in reduced glutathione-BSP conjugation, however, does not explain the rise in conjugated serum bilirubin values during the administration of X-5079C.

Second, the prompt rise in sulfobromophthalein and conjugated bilirubin suggests that X-5079C may have a common action specifically interfering with the excretion of both BSP and bilirubin conjugates. The late rise in unconjugated bilirubin values may be a "backing up" phenomenon secondary to the drug's interference with the excretion of conjugated bilirubin. If this were the case, and the site of action of the drug was placed anatomically at the level of the bile canaliculi, its action would be similar to that of compounds known to produce increased intrabiliary pressure and intrahepatic cholestasis. However, the absence of bile stasis or bile plugging in the canaliculi of the liver sections and the normal alkaline phosphatase and glutamic oxaloacetic transaminase values are against this explanation.

Finally, the drug may produce alterations in BSP and bilirubin metabolism by separate mechanisms, one of which may impede the liver's uptake, conjugation, or excretion of BSP. Further chromatographic analysis of BSP conjugation during the administration of this drug is presently being studied to clarify this mechanism (9). A second and possibly independent mechanism may impede excretion of conjugated bilirubin.

It is frequently difficult to establish the etiologic role of a drug in hepatic injury since the liver participates in many systemic reactions as part of a variety of diseases. These reactions of alteration of parenchymal cells, including focal

TABLE 2

MICROSCOPIC STUDIES OF LIVER SECTIONS IN 14 PATIENTS TREATED WITH ANTIFUNGAL AGENT X-5079C

Pre-treatment				Treatment						Post-treatment				
Patient Diagnosis	Sulfobromophthalein	Conjugated Bilirubin	Microscopic	Daily Dose	Day of Therapy	Sulfobromophthalein	Conjugated Bilirubin	Dose Received to Date	Microscopic	Sulfobromophthalein	Conjugated Bilirubin	Number of Days after Therapy	Total Dose Received	Microscopic
	per cent	*mg./100 ml.*		*mg./kg. body weight*		*per cent*	*mg./100 ml.*	*gm.*		*per cent*	*mg./100 ml.*		*gm.*	
O.B. *Patient 27* Histoplasmosis	N.D.*	N.D.	N.D.	9	7	N.D.	1.8	2.6	Biopsy: mild chronic inflammation, portal areas; focal granuloma formation; no organisms seen	N.D.	0.33	10	3.7	Autopsy: centrolobular congestion (severe); centrolobular hepatic necrosis; mild chronic inflammation portal areas
C.P. *Patient 31*	First course 3	0.06	N.D.	4					N.D.	6	0.04	37	2.1	Biopsy: normal
Maduromycosis	Second course 6	0.04	Biopsy: normal	4	20	34	0.48	5.6	Biopsy: acute focal hepatic necrosis, chronic inflammation portal areas	17	0.14	11	7.8	Autopsy: extensive areas of focal necrosis, chronic inflammation portal areas
K.H. *Patient 43*	First course 4	0.08	N.D.	4					N.D.	5	0.06	89	5.05	Liver biopsy: normal
Aspergillosis	Second course 5	0.06	Biopsy: normal	4	24	26	0.28	6.175	Biopsy: chronic inflammation portal areas	3	0.08	14	15.06	N.D.
C.B. *Patient 46* Histoplasmosis	First course 7	0.06	N.D.	4					N.D.	4	0.03	181	5.3	Biopsy: normal
Aspergillosis	Second course 4	0.03	Biopsy: normal	4	40	54	0.66	8.15	Biopsy: chronic inflammation, portal areas	8	0.08	274	15.2	Biopsy: normal
A.W. *Patient 65* Blastomycosis	First course 12	0.08	Biopsy: normal	4	26	20	0.38	6.93	Biopsy: mild chronic inflammation portal areas	17	0.10	100	10.6	Biopsy: increased inflammation in portal areas; small bile duct proliferation, occasional binucleate hepatic cells

A.W. *Patient 65* Blastomycosis (*cont'd*)	Second course	17	0.10	Biopsy: increased inflammation, portal areas, small bile duct proliferation, occasional binucleate hepatic cells	4	66	45	1.10	18.47	Biopsy: chronic inflammation and focal fatty metamorphosis	6	0.04	307	28.74	Biopsy: mild focal fatty metamorphosis
T.M. *Patient 66* Coccidioidomycosis		13	0.07	Biopsy: severe chronic inflammation portal areas, increased connective tissue in portal areas.	3	37	43	0.77	5.68	Biopsy: increase in chronic inflammation, portal areas	15	0.24	10	11.12	N.D.
						70	53	0.65	11.12	Biopsy: same as previous biopsy					
H.L. *Patient 71* Sporotrichosis		5	0.03	Biopsy: normal	4	29	33	0.99	7.28	Biopsy: chronic inflammation, portal areas, mild	4	0.13		9.685	N.D.
D.R. *Patient 11* Blastomycosis		14	0.03	Biopsy: mild chronic inflammation, portal areas; focal fatty metamorphosis	3	28	67	0.74	5.4	Biopsy: same as pretreatment biopsy	7	0.1		5.7	N.D.
O.B. *Patient 87* Sporotrichosis		11	0.08	Biopsy: moderate fatty infiltration	4					N.D.	4	0.01	102	6.72	Biopsy: same as pretreatment biopsy
O.E. *Patient 83* Blastomycosis		3	0.06	N.D.	4					N.D.	4	0.1	115	11.05	Biopsy: focal chronic inflammation, portal areas
B.A. *Patient 88* Histoplasmosis		38	0.17	Biopsy: normal	4					N.D.	8	0.14	3	22.84	Open biopsy: focal granulomatous inflammation
P.G. *Patient 33* Coccidioidomycosis		2	0.05	N.D.	7	13	57	1.7	4.5	Autopsy: moderate centrolobular congestion					

TABLE 2—*Concluded*

Patient Diagnosis	Pre-treatment			Treatment						Post-treatment				
	Sulfobromophthalein	Conjugated Bilirubin	Microscopic	Daily Dose	Day of Therapy	Sulfobromophthalein	Conjugated Bilirubin	Dose Received to Date	Microscopic	Sulfobromophthalein	Conjugated Bilirubin	Number of Days after Therapy	Total Dose Received	Microscopic
	per cent	*mg./100 ml.*		*mg./kg. body weight*		*per cent*	*mg./100 ml.*	*gm.*		*per cent*	*mg./100 ml.*		*gm.*	
H.D. *Patient 45* *Candida quilliermondii* Endocarditis	6	0.1	N.D.	4					N.D.	17	2.8	3	15.25	Autopsy: severe centrolobular congestion; centro-lobular hepatic necrosis; chronic inflammation portal areas
H.R. *Patient 68* *Candida parapsilosis* Endocarditis	10	0.11	N.D.	3					N.D.	6	0.17	25	4.44	Autopsy: severe centro-lobular congestion; centro-lobular hepatic necrosis; moderate chronic inflammation, portal areas.

* N.D.—not done.

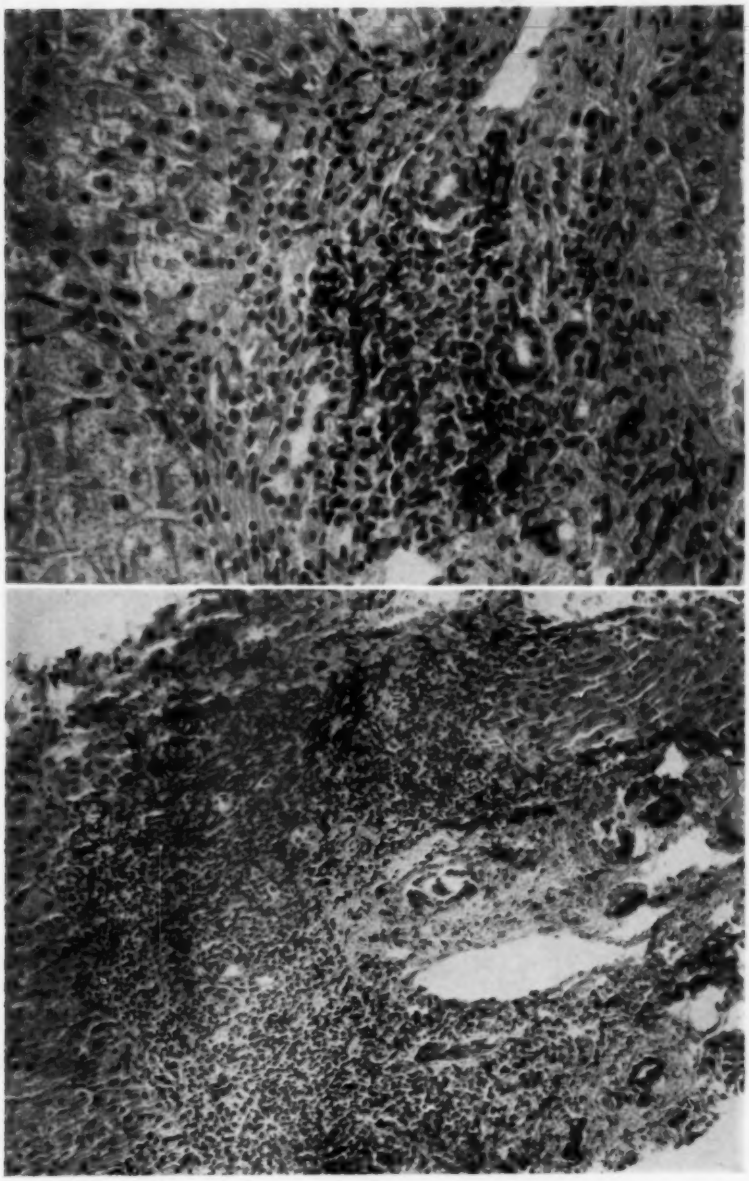

FIG. 3 (Upper). Microscopic section of liver biopsy obtained on thirty-seventh day of therapy with X-5079C (*Patient 66*), showing chronic nonspecific periportal inflammation. (× 290)

FIG. 4 (Lower). Microscopic section of liver biopsy obtained on seventieth day of therapy with X-5079C (*Patient 66*), showing increase in chronic periportal inflammation. (× 150)

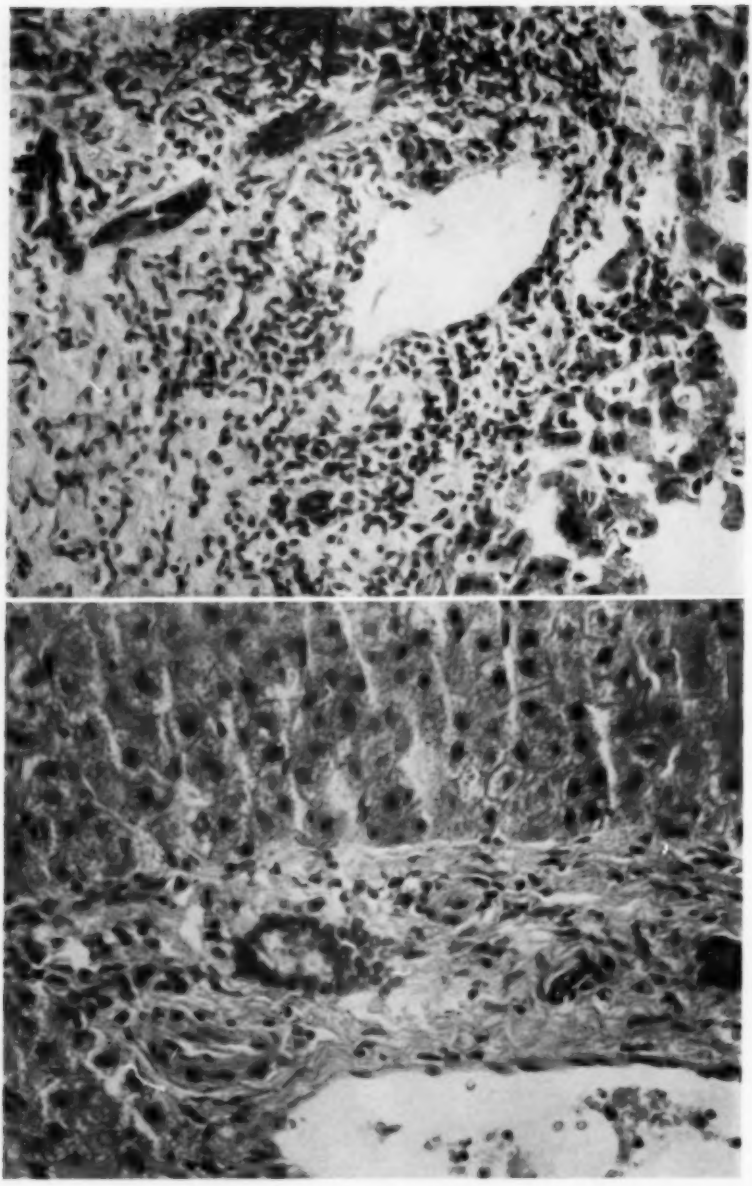

FIG. 5 (Upper). Microscopic section of liver biopsy obtained on fortieth day of therapy with X-5079C (*Patient 46*), showing chronic inflammation in portal areas. (× 290)

FIG. 6 (Lower). Microscopic section of liver biopsy obtained 274 days after administering 15.2 gm. of X-5079C to *Patient 46*, showing normal portal areas. (× 290)

necrosis and mobilization of Kupffer cells, as well as portal inflammation observed in liver biopsy specimens, have been collectively designated as nonspecific reactive hepatitis (10). Hepatic alterations resulting from circulatory disturbances have also been described (11, 12), and the finding of centrolobular necrosis even in biopsy specimens may be a reflection of this and not of a specific drug effect (13). The liver's limited reaction to injury has made it difficult to differentiate the hepatic effect of any one drug.

Attempts have been made, however, to classify drug-induced hepatic injury on the basis of morphologic changes observed in the liver (13). One of the 5 groups of reactions seen frequently is that of *intrahepatic cholestasis* caused by a variety of commonly used therapeutic agents (13, 14). Although chronic periportal inflammation was present in the liver sections of patients receiving X-5079C, eosinophilic infiltration and bile stasis were not seen on any occasion. Focal hepatic cell necrosis was observed in only one biopsy and could not be directly related to the administration of the antifungal agent. Subtle evidence of hepatic cell damage, such as changes in cytoplasmic and nuclear staining and variations of nuclear size seen in many of the biopsies, is not believed to be diagnostic of hepatocellular damage due to drug toxicity. An anatomic cause for the alterations in liver function seen in patients treated with X-5079C is difficult to define. Therefore, it is suggested that this antifungal agent be added, by virtue of the morphologic response produced, to the "unclassified group" of drugs associated with hepatic injury.

Summary

Administration of the antifungal agent X-5079C to 28 patients with systemic fungous disease was associated with an abrupt and substantial rise in sulfobromophthalein retention soon after the start of treatment. Sulfobromophthalein retention was sustained throughout treatment, but fell promptly to normal after cessation of the administration of the drug. Lesser degrees of abnormality were observed in conjugated and unconjugated bilirubin values and some other liver function tests. Microscopic studies of liver sections from 14 patients showed the presence of periportal inflammation which disappeared after treatment was discontinued.

Sumario

Metabolismo Alterado de la Sulfobromoftaleína en el Hombre, Inducido por el Agente Antifungoso X-5079C

La administración del agente antifungoso X-5079C a 28 sujetos que tenían enfermedad fungosa generalizada se asoció con una elevación brusca y sustancial de la retención de la sulfobromoftaleína poco después de iniciarse el tratamiento. La retención de sulfobromoftaleína se sostuvo durante todo el tratamiento, pero bajó prontamente a lo normal después de cesar la administración de X-5079C. Se observaron grados más bajos de anomalía en los valores de bilirrubina conjugada e inconjugada y en algunas otras pruebas de la función hepática. Los estudios microscópicos de cortes hepáticos procedentes de 14 enfermos revelaron la existencia de inflamación periportal que desapareció después de discontinuarse el tratamiento.

Resume

Changement chez l'homme, du métabolisme de la sulfobromophtaléine, suscité par le médicament antifungique X-5079C

L'administration du médicament antifungique X-5079C chez 28 malades ayant une maladie systémique fungique a été associée à une élevation brusque et importante de la rétention de la sulfobromophtaléine aussitôt après le début du traitement. La rétention de sulfobromophtaléine s'est maintenue pendant tout le traitement, mais tomba rapidement jusqu'à la normale après l'arrêt de l'administration de X-5079C. Des degrés moins importants d'anormalité furent observés dans les quantités de bilirubine conjuguée et non conjuguée et dans certains autres tests de la fonction hépatique. Des études microscopiques de coupes de foie, chez 14 malades, montrérent la présence d'une inflammation périportale qui disparut après l'arrêt du traitment.

Acknowledgment

The writers acknowledge the helpful suggestions of Dr. Steven Schenker.

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ACTION OF NUCLEIC ACIDS AND PLANT-GROWTH HORMONES ON THE GROWTH AND VIABILITY OF MYCOBACTERIA[1, 2]

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INTRODUCTION

The first studies from this laboratory (1) on the action of substances of animal origin on the growth of acid-fast bacilli revealed that aging cultures of mycobacteria containing few and slowly growing bacilli are particularly suitable for the study of growth problems. Only these cultures brought out the action of growth substances; in cultures of young, rapidly multiplying bacteria, growth is hardly affected by the presence or absence of a growth substance. This was confirmed recently: in 24-hour-old cultures of *Bacillus globigii* growth stimulation by kinetin, 3-indoleacetic acid, and gibberellic acid failed to produce any observable effect (2).

In a previous paper (3), one of the writers (E. D.) showed the highly stimulating effect of nucleic acids obtained from pathogenic tubercle bacilli on the growth of aging cultures of BCG and tubercle bacilli. Only highly diluted nucleic acids, particularly the dilution 10^{-3} γ per ml., showed growth-stimulating effect. Less diluted substance had an inhibiting effect on growth (4).

The writers' further aim, the subject of this communication, was to investigate the influence of commercial nucleic acids on the growth of BCG and to compare their action with that of some substances known as plant-growth hormones. It is also planned to communicate the results obtained with cultures of tubercle bacilli maintained under paraffin oil over a period of five years and with BCG cultured for two years under paraffin oil in the presence of nucleic acids.

It has recently been shown (5) that ribonucleic acid has a more stimulating effect on the growth of tubercle bacilli than desoxyribonucleic acid. Because of this, only the action of ribonucleic acid on the growth of BCG was investigated in the subsequent work.

MATERIALS AND METHODS

Ribonucleic acid from yeast, kinetin, 3-indoleacetic acid, and gibberellic acid were commercial products.[3] For the purpose of sterilization, 10 mg. of the substance were dissolved or suspended in approximately 2 ml. of ethanol in a sterile tube and dried overnight in a vacuum desiccator. After drying, the substances were dissolved in 0.01 M phosphate buffer of pH 7.0 and, in the appropriate concentrations, added to Löwenstein-Jensen medium before inspissation. The medium was then dispensed in Pyrex screw-cap tubes (150 by 20 mm.) and heated in a dry oven at 92°C. for forty-five minutes.

The action of these substances on the growth of BCG was tested in two concentrations and in four BCG cultures of different ages. The first series of experiments was carried out with a medium containing 0.05 γ of substance per ml.; in the second series, a medium containing 0.025 γ per ml. was used.

BCG cultures were grown for ten days at 37°C. on Löwenstein-Jensen medium and then kept in a refrigerator at 5°C. for twenty, ten, seven, and three months, respectively. The twenty-month-old cultures did not grow when transplanted on Löwenstein-Jensen medium after six weeks of incubation; the ten-month-old ones produced few colonies; the seven-month-old ones formed isolated colonies all over the medium; the three-month-old cultures showed good but retarded growth. In the latter group colonies appeared after two weeks of incubation, while in fresh cultures colonies developed in the first week.

The method of standardization of bacterial suspensions, inoculation technique, and composition of medium were described in a previous report from this laboratory (3). Ten tubes of Löwenstein-Jensen medium containing 0.05, 10 tubes containing 0.025 γ of growth substance per ml., and 20 controls were inoculated with twenty-, ten-, seven-, and three-month-old BCG cultures, respectively. Experiments were repeated twice with four growth substances. A total of 640 tubes containing growth substances and 640 controls were inoculated and incubated for six weeks at 37°C.

Before these experiments on solid medium were started, the growth of tubercle bacilli and BCG

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[2] This investigation was supported by research grant E-2699(C1) from the Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[3] Nutritional Biochemicals Corporation, Cleveland, Ohio.

was tested on liquid medium under paraffin oil. On October 21, 1955, five 50-ml. Pyrex Erlenmeyer flasks containing 20 ml. of liquid medium were inoculated with a ten-day-old pellicle, approximately 0.4 cm.^{2} in size, of the pathogenic H37Rv strain of tubercle bacilli. The surface of these flasks was covered with a 1.0-cm. thick layer of sterile paraffin oil.[4] At the moment of contact between the oil and the pellicle, bacilli were forcibly dispersed in the interface of oil and liquid medium. These cultures were incubated at 37°C. uninterruptedly until October 21, 1960 (five-year cultures). The pathogenicity of these cultures was tested by inoculation in guinea pigs in the first year, the second year, and after five years of incubation.

The experiments with the five-year H37Rv cultures led to a second series of experiments with BCG. On November 7, 1958, 10^{-3} γ of nucleic acids per ml., isolated from pathogenic tubercle bacilli, were added to five Pyrex screw-cap tubes (150 by 25 mm.) containing 10 ml. of liquid medium.[5] The surface of these tubes, as well as that of five controls without nucleic acids, was inoculated with one loop (approximately 0.3 cm.^{2} in size) of BCG pellicle grown for ten days on liquid medium. Six of these tubes were covered with a 1.0-cm. thick layer of sterile paraffin oil; the other four were covered with a 1.3-cm. thick layer. Five other tubes containing liquid medium with nucleic acids were inoculated in the same way and covered with oil; thereafter they were connected with a vacuum pump and evacuated until a 100 μ vacuum was reached. At that moment they were hermetically sealed by means of a blast lamp. All inoculated tubes were incubated uninterruptedly at 37°C. until November 7, 1960 (two-year cultures). On that day the viability of these cultures, with the exception of evacuated ones, was tested. Two drops (0.08 ml.) from the deposits of nucleic acid cultures were planted on each of five tubes of Löwenstein-Jensen medium, and two drops from the deposite of controls were planted on each of five other tubes. The whole sediment of two controls was collected and inoculated on five Löwenstein-Jensen tubes. The paraffin layer of two nucleic acid cultures and of two controls was skimmed, and thereafter the cultures were centrifuged for thirty minutes at 7,000 r.p.m., washed three times with distilled water, dried in vacuum over calcium chloride, and weighed.

Results

Löwenstein-Jensen medium, nucleic acids: Of a total of 40 tubes inoculated with twenty-month-old BCG cultures, three tubes, two of them containing 0.05 γ and the third 0.025 γ of nucleic acids per ml., produced cultures after six weeks of incubation; 40 controls without nucleic acids failed to produce cultures. The nucleic acid medium inoculated with ten-month-old BCG showed signs of growth within twenty days, while only 26 of the 40 controls produced cultures. The results yielded by seven-month-old bacilli were similar to ten-month-old bacilli, but the first colonies appeared in twelve days, and 36 controls were positive. All tubes inoculated with three-month-old bacilli produced cultures, but the growth on nucleic acid medium started five days earlier than on medium without acids. Growth on nucleic acid medium was approximately twice as abundant as on medium without nucleic acids.

Liquid medium, growth under paraffin oil: Growth proceeds slowly on the bottom of the flasks. In five years strain H37Rv produced large masses of yellowish culture which consisted of long cords of acid-fast bacilli. In the first two years, these bacilli grew on Löwenstein-Jensen medium and were pathogenic for guinea pigs. Thereafter, their viability and pathogenicity decreased. In the fifth year, when inoculated on Löwenstein-Jensen medium, these bacilli did not develop cultures after six weeks of incubation. Inoculated subcutaneously in 2 guinea pigs, the bacilli produced small nodules at the site of inoculation which disappeared in two weeks. In the fifth year the bacilli were dead.

Liquid medium, growth under paraffin oil in the presence of nucleic acids: BCG cultures grown for two years under these conditions developed a 1.0-cm. thick granular mass on the bottom of the tubes; the controls, without nucleic acids, formed a thin pellicle in the same area (figure 1). BCG grown under paraffin oil in the presence of added nucleic acids consisted of well-developed cords of acid-fast bacilli; the controls contained short acid-fast strands and scattered islets of acid-fast bacilli embedded in cyanophil substance. On Löwenstein-Jensen medium two drops of nucleic acid cultures produced a great number of colonies after ten days of incubation at 37°C.; the medium inoculated with the sediment of a control did not show any growth after five weeks of incubation. The whole sediment of two controls after five weeks of incubation produced three small colonies of acid-fast bacilli (figure 2).

The average weight of a nucleic acid culture deposit was 0.0164 gm.; that of the control, 0.0012 gm.

Cultures in evacuated tubes did not form a pellicle in the interface or sediment.

[4] Mallinckrodt Products.

[5] The technique of isolation and identification of nucleic acids from tubercle bacilli was given in a previous paper (3).

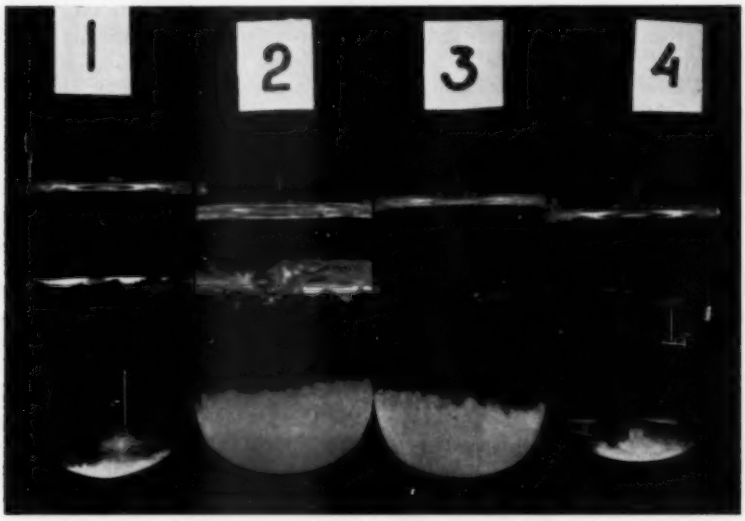

FIG. 1. BCG cultures grown uninterruptedly for two years in liquid medium at 37°C. under paraffin oil. Tubes 2 and 3 were cultured in the presence of 10^{-3} γ of nucleic acid per ml.; tubes 1 and 4 were grown without nucleic acids; tubes 2 and 4 contained a 1.0-cm. thick layer of paraffin oil; tubes 1 and 3 contained a 1.3 cm. thick layer of paraffin oil.

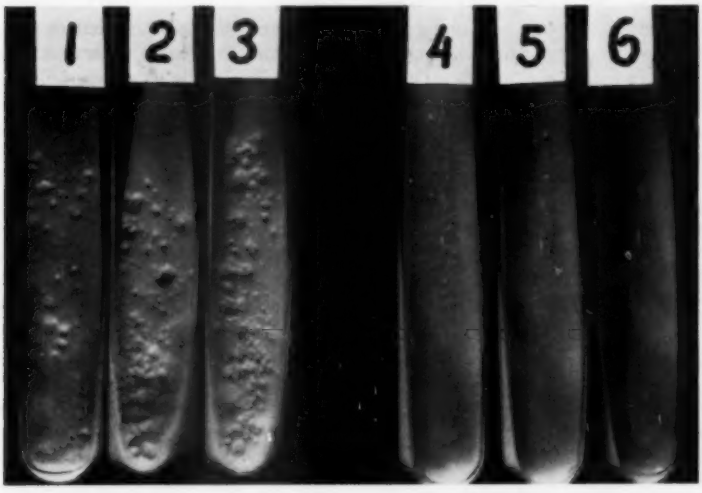

FIG. 2. BCG cultures on Löwenstein-Jensen medium. Tubes 1, 2, and 3 were inoculated with two drops (0.08 ml.) of BCG cultures grown uninterruptedly for two years in liquid medium under paraffin oil at 37°C. in the presence of 10^{-3} γ of nucleic acid per ml. These tubes were incubated for ten days at 37°C. Tubes 4, 5, and 6 were inoculated with the whole sediment of two controls grown uninterruptedly for two years in liquid medium at 37°C. in the absence of nucleic acids. These tubes were incubated for five weeks at 37°C.

Kinetin, 6-furfurylaminopurine, $C_{10}H_9N_5O$, is a degradation product of nucleic acids and has been recognized as a growth factor in higher plants (6, 7). A Löwenstein-Jensen medium containing 0.05 and 0.025 γ of this substance per ml., when inoculated with twenty-month-old BCG, failed to produce cultures; media inoculated with ten-month-old bacilli, on the other hand, all produced colonies, but only after twenty-four days of incubation; 28 of 40 controls produced colonies. Seven- and three-month-old bacilli showed the same amount of growth as medium containing ribonucleic acid.

3-Indoleacetic acid, $C_{10}H_9NO_2$, known as auxin in higher plants, did not induce growth in twenty-month-old bacilli, but stimulated the growth of ten-, seven-, and three-month-old bacilli almost as intensely as ribonucleic acid. Media containing 3-indoleacetic acid produced a larger amount of bacillary mass than controls.

Gibberellic acid, $C_{19}H_{22}O_6$, was isolated from the fungus *Gibberella fujikuroi* by the Japanese investigator Kurosava and co-workers (8). The fungus produced increased growth in infected rice seedlings. It was observed that this substance promotes the growth and respiration of tubercle bacilli (8). No growth was obtained on Löwenstein-Jensen medium containing 0.05 or 0.025 γ of this substance per ml. when inoculated with twenty-month-old bacilli. Of 40 tubes inoculated with ten-month-old bacilli, 32 produced cultures after six weeks of incubation. In seven- and three-month-old bacilli, growth stimulation by this substance was less vigorous than in nucleic acids, kinetin, and 3-indoleacetic acid (table 1).

TABLE 1

GROWTH OF BCG ON LÖWENSTEIN-JENSEN MEDIUM AND IN A LIQUID MEDIUM IN THE PRESENCE OF GROWTH SUBSTANCES

Growth Substance Added	Age of BCG Löwenstein-Jensen Medium					BCG Growth 20 Months, under Paraffin Oil Liquid Medium	
	10 Days	3 Months	7 Months	10 Months	20 Months	Nucleic Acids Added	Without Nucleic Acids
Nucleic acids	xxxx	xxx	xx	xx	x	xxx	3 colonies
Kinetin	xxxx	xxx	xx	x	—		
3-Indoleacetic acid	xxxx	xxx	xx	x	—		
Gibberellic acid	xxxx	xx	x	x	—		
Controls	xxx	xx	x	x	—		

Number of crosses indicates amount of growth.

DISCUSSION

Two decades ago Caspersson and his co-workers (9) observed that the aging of bacterial cells runs parallel to loss of colorability of cytoplasm, a process caused by the decrease in the ribonucleic acid content of the cells. When the growth of *Escherichia coli*, yeast, and rabbit macrophages stops, ribonucleic acid disappears from the cells (10). The growth of the cells starts with synthesis of ribonucleic acid. This substance of bacteria is very similar to that of yeast, higher plants, and animal cells (11). These findings may explain why commercial ribonucleic acid obtained from yeast has a powerful growth-stimulating effect on aging acid-fast bacilli; 3-indoleacetic acid induces division of chromosomes in resting nuclei and causes increased growth in higher plants (12, 13). The same mechanism may be at play in the growth stimulation of BCG cells by ribonucleic acid and kinetin.

None of the plant growth hormones investigated had as great a growth-stimulating effect on the BCG cells as ribonucleic acid. Stimulation induced by kinetin is nearly as strong as that induced by ribonucleic acid, but it should be remembered that the ribonucleic acid which was used came from a relatively crude commercial preparation, while kinetin was a chemically pure substance. Because of this, the growth-stimulating effect of kinetin must be inferior to that of ribonucleic acid.

Pathogenic H37Rv tubercle bacilli in liquid medium under paraffin oil produced abundant culture and were pathogenic for guinea pigs for two years, but died subsequently. Two-year-old BCG cultures in liquid medium under paraffin oil in the presence of a small amount of nucleic acids stayed alive and produced approximately fourteen times more bacillary mass than bacilli grown without nucleic acids; most of the bacilli grown without nucleic acids were dead at that time. The liquid medium used in the present experiments contains 10 per cent bovine serum and 0.5 per cent peptone. These substances contain ample amounts of nucleic acids and nucleoproteins; notwithstanding this, BCG bacilli under paraffin oil were unable to utilize these sub-

stances. Evidently, under those conditions, the possibility of assimilating nutrients necessary to synthesize nucleic acids or to split nucleoproteins was impaired, and life slowed down until the cells died. Small amounts of free nucleic acids added to the medium preserved life under restricted air conditions.

At low oxygen tension, BCG cells did not grow in the presence of added nucleic acids, but a trace of oxygen present in nonevacuated medium was enough to induce in them slow uninterrupted growth for two years. Only three sources of oxygen were available to the bacilli under paraffin oil: the oxygen dissolved in the medium, chemically bound oxygen, and that which could penetrate through paraffin oil. Without added nucleic acids the amounts of oxygen provided by these sources were not sufficient to sustain life.

Summary

The action of the plant-growth hormones, kinetin, 3-indoleacetic acid, and gibberellic acid, was compared with growth stimulation by commercial ribonucleic acid in aging BCG cultures kept in a refrigerator at 5°C. for twenty, ten, seven, and three months. A total of 1,280 tubes were inoculated with these cultures. The highest growth stimulation was shown by ribonucleic acid.

The pathogenic strain H37Rv was grown in liquid medium for five years at 37°C. under paraffin oil. A large mass of bacilli was formed which remained pathogenic for two years; after five years these cultures were dead.

BCG was grown uninterruptedly for two years in liquid medium under paraffin oil in the presence of 10^{-3} γ of nucleic acid per ml. The controls were grown without nucleic acid. After two years the cultures containing nucleic acid had developed an abundant mass of acid-fast bacilli, while the controls showed only scanty growth. The BCG grown in the presence of nucleic acid produced approximately fourteen times more dry bacillary masses than the controls. On Löwenstein-Jensen medium the bacilli from the nucleic-acid cultures showed abundant growth in ten days, while the bacilli grown in the absence of nucleic acid developed three small colonies in five weeks. The BCG cultures in liquid medium under paraffin oil in evacuated tubes did not grow.

This phenomenon of conservation of life under paraffin oil in the presence of nucleic acid provides a method of ensuring the survival of cultures of BCG and other mycobacteria over long periods of time.

Sumario

Efecto de los Acidos Nucleicos y de las Hormonas que Hacen Crecer las Plantas sobre el Crecimiento y la Viabilidad de las Micobacterias

Se comparó el efecto de las hormonas que fomentan el desarrollo de las plantas, kinetina, ácido 3-indoleacético y ácido gibberélico, con la excitación del desarrollo producida por el ácido ribonucleico del comercio, en lo tocante a madurar cultivos de BCG mantenidos en un refrigerador a 5°C. durante veinte, diez, siete y tres meses. Con estos cultivos se inoculó un total de 1,280 tubos. La mayor excitación del desarrollo correspondió al ácido ribonucleico.

La cepa patógena H37Rv fué cultivada en medio líquido por cinco años a 37°C., bajo aceite de parafina. Se formó así una gran masa de bacilos que permanecieron patógenos durante dos años; al cabo de cinco años estos bacilos habían muerto.

El BCG fué cultivado sin interrupción por dos años en medio líquido bajo aceite de parafina en presencia de 10^{-3} γ de ácido nucleico por ml. Se cultivaron los testigos sin ácido nucleico. Al cabo de dos años, los cultivos que contenían ácido nucleico habían formado una masa abundante de bacilos ácidorresistentes, mientras que los testigos no revelaban más que una proliferación escasa. Los BCG cultivados en presencia de ácido nucleico produjeron aproximadamente catorce veces más aglomeraciones bacilares secas que los testigos. En el medio de Löwenstein-Jensen los bacilos derivados de los cultivos con ácido nucleico revelaron abundante proliferación en diez días, en tanto que los cultivados sin ácido nucleico manifestaron tres pequeñas colonias en cinco semanas. Los BCG cultivados en medio líquido bajo aceite de parafina en tubos vaciados no proliferaron.

Este fenómeno de conservación de la vida bajo aceite de parafina en presencia de ácido nucleico suministra un método que garantiza la sobrevivencia de cultivos del BCG y de otras micobacterias durante períodos prolongados de tiempo.

Resume

Action des acides nucléiques phytohormones sur le développement et la viabilité des mycobactéries

L'action des phytohormones, la kinétine, l'acide 3-indolacétique, et l'acide gibberellique, a été comparée à la stimulation du développement par l'acide ribonucléique commercial, dans des cul-

tures agées de BCG, gardées dans un réfrigérateur à 5°C., pendant vingt, dix, et trois mois. On a inoculé un total de 1,280 tubes avec ces cultures. La plus forte stimulation de développement se montra avec l'acide ribonucléique.

On fit pousser la souche pathogène H37Rv dans un milieu liquide pendant cinq ans à 37°C., sous huile de paraffine. Une grande quantité de bacilles se formèrent et restèrent pathogènes pendant deux ans; après cinq ans ces cultures moururent.

Les BCG a été cultivé sans interruption pendant deux ans dans le milieu liquide sous huile de paraffine en présence de 10^{-3} γ d'acide nucléique par ml. Après deux ans les cultures contenant de l'acide nucléique développèrent une quantité abondante de bacilles acido-résistants, tandis que les contrôles montrèrent seulement un développement minimal. Les BCG poussés en présence d'acide nucléique produisirent à peu près quatorze fois plus de quantités bacillaires sèches que les contrôles. En milieu de Löwenstein-Jensen, les bacilles des cultures avec acide nucléique montrèrent une culture abondante en dix jours, tandis que les bacilles poussés sans acide nucléique développèrent trois petites colonies en cinq semaines. Les cultures de BCG poussées en milieu liquide sous huile de paraffine, dans des tubes sous vide, ne poussèrent pas.

Ce phénomène de conservation de la vie sous huile de paraffine, en présence d'acide nucléique fournit une méthode pour assurer la survie des cultures de BCG et d'autres mycobactéries pendant longtemps.

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PULMONARY RESECTIONS IN PATIENTS HARBORING DRUG-RESISTANT TUBERCLE BACILLI[1, 2]

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Introduction

Although the treatment of pulmonary tuberculosis with combinations of streptomycin, para-aminosalicylic acid (PAS), and isoniazid has been amazingly successful, an increasing reservoir of patients harboring resistant organisms has accumulated whose disease is difficult to treat. The results of medical regimens including the so-called "secondary" drugs (2-amino-5-sulfanilylthiazole (Promizole®), pyrazinamide, amithiozone, oxytetracycline, viomycin, cycloserine, and kanamycin) have been disappointing (1). The use of surgery in such patients has met with many problems, but has provided the best results. It is the purpose of this communication to present the writer's surgical experience over a ten-year period.

Materials and Methods

Between January 1, 1950, and January 1, 1960, 265 pulmonary resections were performed upon 220 patients harboring tubercle bacilli resistant to 10γ of streptomycin per ml., 10γ of PAS per ml., and/or 1γ of isoniazid per ml. Susceptibility studies were performed as reported previously (2). Two hundred and thirty-seven operations were performed in patients with streptomycin-resistant organisms, 178 in patients with PAS-resistant organisms, and 158 in patients with isoniazid-resistant organisms. In 68 operations the patients' organisms were resistant to one drug, in 91 they were resistant to two drugs, and in 106 they were resistant to three drugs. Culturable organisms were present in 197 instances either in the sputum at the time of surgery or in the resected tissue. The disease was far advanced[3] in 147 instances, moderately advanced in 114, and minimal in 4. Pneumonectomy was performed in 20 instances, lobectomy in 52, segmental resection in 12, wedge resection in 84, and combinations of procedures were employed in 97. Whenever possible all palpable disease was removed, but this was rarely the case. Usually only the cavity-bearing portion of the lung was removed, and foci of nodular disease remained.

If "adequate" drug therapy is defined as the equivalent of one year of continuous treatment with combinations of streptomycin, PAS, and/or isonizid, then only 25 resections were accompanied by adequate therapy. The remainder of the patients were treated principally with various combinations of the secondary drugs. Whenever organisms were known to be susceptible to streptomycin, PAS, or isoniazid, one of those drugs was prescribed. Early in this series a few operations were performed without effective drug coverage but, because of poor results, this practice was discontinued.

Early tuberculous complications occurring in the first postoperative month included empyema, spread of disease, wound infection, and persistence of viable tubercle bacilli in the expectorations. Tuberculous complications of a similar nature appearing or persisting after the first postoperative month are referred to as "late."

Results

Tuberculous complications (table 1) occurred in 62 (23.4 per cent) of the patients who had resections. Most of the complications did not respond favorably to further drug therapy and required additional surgery in the form of thoracoplasty or resection.

Three (12 per cent) of 25 resections accompanied by adequate chemotherapy were followed by tuberculous complications, compared with the occurrence of 59 complications (25 per cent) after the 240 resections performed without such coverage. The 3 patients who suffered complications in spite of adequate therapy had been treated previously. It is possible that their organisms might not have proved susceptible to slightly lower concentration of drugs *in vitro*. However, the complications were not serious, as they consisted of transient bacteriologic relapses.

The complication rate was undoubtedly influenced unfavorably by extensive disease. Twenty-nine per cent (42) of the resections in patients with far advanced disease were followed by tuberculous complications, compared with 18 per cent (20) in patients with moderately ad-

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] Presented before the Medical Session, as part of *Section 7A*, at the annual meeting of the American Thoracic Society, Cincinnati, Ohio, May 24, 1961.

[3] Classified according to the Diagnostic Standards and Classification of Tuberculosis, National Tuberculosis Association, New York, 1955.

TABLE 1

TUBERCULOUS COMPLICATIONS

	Number of Operations	Drug Therapy		Extent of Disease			Viable Organisms	Operations*					Drug Resistance		
		Adequate	Inadequate	Minimal	Moderately Advanced	Far Advanced		P	L	S	W	C	1†	2	3
No complications	203	22	181	4	94	105	139	12	43	10	71	67	55	75	73
Tuberculous complications	62	3	59	0	20	42	58	8	9	2	13	30	13	16	33
Total	265	25	240	4	114	147	197	20	52	12	84	97	68	91	106

* P = Pneumonectomy; L = Lobectomy; S = Segmental resection; W = Wedge resection; C = Combination of procedures.

† Numerals indicate resistance to one, two, or three drugs.

vanced disease and none in those with minimal disease.

Twenty-nine per cent (58) of 197 resections in patients who harbored culturable organisms either in sputum at the time of surgery or in resected tissue were followed by tuberculous complications, compared with 6 per cent (4) complications after 68 resections in patients in whom tubercle bacilli were not demonstrated. Tuberculous complications developed in 13 (19 per cent) of 68 resections performed in the presence of organisms resistant to one drug, 16 (18 per cent) of 91 performed in the presence of organisms resistant to two drugs, and 33 (31 per cent) of 106 performed in the presence of organisms resistant to three drugs. The relatively poor results in the group with tubercle bacilli resistant to three drugs probably reflect not only the extensive disease but also the fact that in this group none of the major drugs (streptomycin, PAS, and isoniazid) were available for use.

Tuberculous complications occurred after 8 (40 per cent) of 20 pneumonectomies, 9 (17 per cent) of 52 lobectomies, 2 (17 per cent) of 12 segmental resections, 13 (15 per cent) of 84 wedge resections, and 30 (31 per cent) of 97 combined procedures. The high incidence of complications in the pneumonectomy and combined procedure groups undoubtedly reflects the extensive disease in addition to the more frequent occurrence of "space problems." It is interesting that, contrary to general opinion, wedge resection in the presence of extensive disease yielded excellent results.

Of the 220 patients in this series, 184 (83.6 per cent) are living and noninfectious, 16 (7.3 per cent) are living and infectious, and 20 (9.1 per cent) are dead. Some of the patients who are now infectious may be aided by further surgery. The principal causes of death are listed in table 2. Actually, 6 patients were noninfectious at the time of death. An additional 6 patients died of causes unrelated to tuberculosis even though they were still infectious. Only 3 patients died as the result of progressive disease. The largest single cause of death was cardiopulmonary insufficiency, which is not surprising considering the poor condition of many of the patients prior to surgery.

TABLE 2

DEATHS

Principal Cause	Infectious	Noninfectious	Early	Late	Total
Progressive disease	3			3	3
Cardiopulmonary insufficiency	5	2	1	6	7
Bleedinggastric ulcer	1			1	1
Toxic hepatitis	1	1	1	1	2
Coronary occlusion		1		1	1
Uremia	1			1	1
Cardiac arrest	1		1		1
Leukemia		1		1	1
Glomerulonephritis		1		1	1
Surgery (elsewhere)	1			1	1
Undetermined	1		1		1
					20

DISCUSSION

If a patient harbors organisms resistant to streptomycin, PAS, or isoniazid, he may be treated successfully without surgery provided

he receives continuous combined therapy with the other two drugs for at least a year. Since most such patients have been previously treated unsuccessfully, and since *in vitro* studies do not detect the presence of organisms resistant to slightly lower concentrations of drug than those employed in the laboratory, results as good as those achieved with original treatment cannot be expected. For this reason surgery should be prescribed more liberally, especially when cavitary disease is present.

When the patient harbors tubercle bacilli resistant to two or more of the major drugs, experience has indicated that there is little hope for cure unless surgery is performed. Pulmonary resection is the procedure of choice in the majority of cases. Secondary drugs yield good results with pulmonary resection when two or more are administered for approximately a year. Pulmonary resection is hazardous in the absence of therapy with drugs to which the patient's organisms are susceptible.

Although extent of disease, bacteriologic status of sputum, drug therapy, and type of surgery influence results, one of the greatest problems in the drug-resistant group is poor patient cooperation which, incidentally, is frequently the fundamental reason for the presence of resistant organisms and advanced uncontrolled disease. Because most of the patients with resistant organisms are unreliable, and because "secondary" drugs are toxic, good results can be expected only in a carefully supervised hospital.

Summary

Over a period of ten years, 265 pulmonary resections were performed on 220 patients harboring tubercle bacilli resistant to streptomycin, PAS, and/or isoniazid. Important factors influencing results were drug therapy, extent of disease, presence of culturable tubercle bacilli, type of surgery, and patient cooperation. Tuberculous complications occurred in 23.4 per cent of the patients who underwent resection. The total mortality was 9.1 per cent. Eighty-three and six-tenths per cent of the patients are living and well.

Sumario

Resecciones Pulmonares en Enfermos que Albergan Bacilos Tuberculosos Fármaco-Resistentes

En un período de diez años, se ejecutaron 265 resecciones pulmonares en 220 enfermos que albergaban bacilos tuberculosos resistentes a la estreptomicina, al PAS y/o a la isoniacida. Factores importantes que afectaron los resultados fueron la fármacoterapia, la extensión de la enfermedad, la culturabilidad de los bacilos tuberculosos, la forma de la cirugía y la cooperación del enfermo. Hubo complicaciones tuberculosas en 23.4 por ciento de los enfermos sometidos a la resección. La mortalidad fué de 9.1 por ciento. Ochenta y tres y seis décimas por ciento de los enfermos se encuentran vivos y sanos.

Resume

Resections pulmonaires chez des malades ayant des bacilles tuberculeux résistant aux medicaments

Les auteurs ont effectué, pendant une période de dix ans, 265 résections pulmonaires chez 220 malades ayant des bacilles tuberculeux résistant à la streptomycine, au PAS, et/ou à l'isoniazide. Des facteurs importants influençant les résultats furent la thérapeutique médicamenteuse, l'étendue de la maladie, la possibilité de culture des bacilles tuberculeux, le genre de chirurgie et la coopération du malade. Des complications tuberculeuses se produisirent chez 23.4% des malades qui subirent la résection. La mortalité totale fut de 9,1%. Quatre-vingt-trois et six dixièmes pour cent des malades sont vivants et bien portants.

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THE NATURAL HISTORY OF TUBERCULOSIS IN THE HUMAN BODY[1, 2]

III. Tuberculous Women and Their Children

J. ARTHUR MYERS

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Introduction

Few aspects of any disease have been discussed longer, more frequently, and more extensively than tuberculosis and pregnancy. Apparently the earliest recorded writings are those of Hippocrates (460 to 376 B.C.). For many centuries thereafter, clinicians believed pregnancy and delivery were beneficial to the tuberculous woman; so much so that girls found to have tuberculosis were advised to marry on the ground that pregnancy was good treatment.

It was only slightly more than 100 years ago when Grisolle (1) reported observations indicating that tuberculous lesions worsened during pregnancy. He apparently attributed no detrimental effect to delivery. Thereafter, a prolonged controversy was waged in the literature between those with these opposing beliefs. Some physicians stated that a tuberculous girl could tolerate one pregnancy well, a second maybe, and a third never.

The majority opinion was soon on the side of the deleterious effect. Indeed, the pendulum had swung so far that Pankow (1923) (2) considered interruption of pregnancy obligatory for tuberculous women. In 1927, Rist (3) was strongly of the opinion that pregnancy results in more pronounced symptoms and spread of the tuberculous lesions along with the appearance of new lesions and reactivation of quiescent ones.

In the early 1930's, a preponderance of physicians were still of the opinion that pregnancy is a serious complication of tuberculosis. However, as well documented studies were made, including those by Jennings, Mariette, and Litzenberg (4), more and more reports indicated that usually pregnancy does not have a deleterious effect. In 1948, McKelvey (5) summed up the recent literature which, combined with his own experience, led him to conclude that pregnancy usually has no deleterious effect on tuberculosis.

In 1953, an excellent report was made by Hedvall (6) including a detailed historical review and a careful analysis of 250 tuberculous pregnant women. Although a few lesions worsened during pregnancy, more improved, while the disease in most of the patients remained stationary. No satisfactory evidence was obtained to prove conclusively that pregnancy was responsible for improvement or worsening of tuberculous lesions.

Pregnancy is a natural phenomenon. Tuberculosis is often a lifetime disease; therefore, long-term observations on the same women are important to determine whether pregnancy on one or more occasions may be responsible for the evolution of clinical lesions or may aggravate those already present. An exceedingly practical consideration is whether women who have well-controlled clinical disease are endangered by subsequent pregnancies.

Clinical tuberculosis is a deceiving disease inasmuch as it often passes through a long silent period, continuously or intermittently progressing. It is usually in an advanced stage by the time it manifests itself through symptoms. If symptoms first appear during pregnancy or soon after delivery, it might seem logical to blame this natural phenomenon for the development of the disease. However, with better understanding of the natural history of tuberculosis in the human body regarding insidiousness, periodic examinations must be demanded before, during, and after pregnancy in order to draw dependable conclusions.

The writer's interest in the possible effects of pregnancy on tuberculosis began in 1920 when it was almost commonplace for young women pregnant out of wedlock to request examina-

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[2] Prepared with the aid of the H. Longstreet Taylor Tuberculosis Research Fund provided by the Minnesota Tuberculosis and Health Association; the J. Arthur Myers Research Fund of the Greater University Fund, University of Minnesota; and a bequest by Joseph E. Stennes.

tions, with the hope that tuberculosis might be found to justify legal termination. There was also a considerable number of tuberculous women legitimately pregnant who had such fear of aggravation or reactivation of tuberculosis as to insist upon termination. These were exceedingly unpleasant situations for the physician. Hence, in subsequent years observations were made on pregnant women in whom pregnancy was not terminated artificially, as well as on a limited number in whom pregnancy was terminated.

There was also the question of tuberculosis among the children of tuberculous mothers. Many women were fearful of bearing children because of the prevalent belief in inheritance of the disease. Others believed it would be impossible to protect their children against contagion.

In 1921, an opportunity came to examine a sizeable number of children each year and, when indicated, to make periodic examinations as often and for as long as desired (7). The children examined ranged in age from early infancy through high school. About the same time, an opportunity was afforded to make long-term studies on university students.

A study on tuberculous pregnant women was undertaken in 1934 as a cooperative activity between chest clinics and obstetrical services which provided information concerning tuberculosis in pregnant women and methods of protecting their children from infection (8).

The present report is based largely on tuberculous women who experienced one or more pregnancies and were examined or treated through a private office in association with sanatoriums, hospitals, clinics, and other physicians.

Such a study could not be initiated today and there will not be an opportunity to make such observations again in this area because of the widespread use of antituberculous drugs which often interrupt the natural history of tuberculosis in the human body. Moreover, it probably will never again be possible to make such observations on children of tuberculous mothers because of the administration of antituberculous drugs prophylactically to babies of tuberculous mothers and, in other cases, therapeutically to children who become tuberculin reactors.

Material and Methods

Persons included in this report consist of 404 women seen between 1920 and 1955 who had diagnoses of clinical pulmonary tuberculosis before, at the time of, or after the initial examination. These women had one to eight pregnancies before the original diagnosis was made, and during or after treatment. Although contact has been continued with a good many of them, others had to be traced, often with much difficulty, to various states and some to other countries in order to obtain current information concerning the tuberculosis status since they were last examined or treated here.

Women who were elderly on first examination are included since they provided important histories with reference to tuberculosis in their families and especially the amount of destruction among their children. Several of the mothers were first seen as children or university students, or both, and periodic observations continued through marriage and motherhood.

The 872 children of these women have been followed with reference to acquired infection and development of clinical disease. This was done in order to determine the effectiveness of the methods designed to protect against invasions of tubercle bacilli and to establish a prognostic baseline among children of tuberculous mothers.

Testing was done with original tuberculin. In the early 1920's, the cutaneous scarification method of administration of Pirquet was employed, both for mothers and children. Special studies revealed that the puncture method of Stewart (9) produced the same results as the Pirquet scarification, and the intracutaneous method of Mantoux was only slightly more efficacious than the Pirquet method (10). With the adoption of the intracutaneous method in the 1920's, the initial dose consisted of 0.1 mg., and for nonreactors a second dose of 1.0 mg. In some of the later testing of children, 5 tuberculin units and, in others, 1.0 mg. of tuberculin was employed as a single test dose. Induration or edema of 5 mm. or more in diameter was regarded as a reaction. Families which have moved away have had their testing done by local physicians, practically all of which was by the intracutaneous method.

Observations

It has been impossible to contact 16 of the 404 women included in this study. Of the 388 who have been followed here continuously or recently traced, 72 have died.

Deaths from Nontuberculous Conditions

Death was caused by nontuberculous conditions in 21 who were examined here between 1920 and 1948: fifteen housewives, one maid, three nurses, one student, and one teacher. Twelve gave no history of contact with contagious cases of tuberculosis. Four had been exposed to tuberculous members of the immediate family, three to patients as student nurses, one

to a fellow tuberculous worker, and one in a home where she worked as a maid. Eighteen had advanced disease, and tubercle bacilli were recovered from 17.

The average age of these 21 women was 28.5 years at the time of the first examination and the age range was 18 to 55 years. At the time of death, the age range was 32 to 72 years and the average was 47.6 years. Only one patient had attained the age of 40 years or older on the first examination, but 14 others were in that age bracket before death. The period of observation extended over 454 person-life years.

All of the subjects were leading active lives and were free from progressive clinical tuberculous disease when they died from the following: 4, coronary thrombosis; 4, cor pulmonale; 3, cerebral hemorrhage; 3, carcinoma of the breast; 2, lymphatic leukemia (one at 32, the other at 34 years of age); one, ruptured myocardial infarct; one, spontaneous pneumothorax; one, barbiturate poisoning; one, murder; and one, suicide.

These 21 women had 34 children, 12 of whom were born before the mother's tuberculosis was known to exist, 4 during treatment, and 18 later. One had been in a sanatorium in 1925 for 17 months with a diagnosis of tuberculous enteritis complicating pulmonary disease. Enteritis was then considered such a fatal condition that the mother had five of the next six pregnancies terminated between 1927 and 1944. One was allowed to go to term in 1937. There was no other termination of pregnancy reported in this group.

Of the 12 births which occurred before the mothers' disease was detected, only 2 occurred within several years of pregnancy. One woman delivered within one year of the diagnosis of tuberculosis, but no examination had been made before the baby was born. The other diagnosis was made about the time of delivery, and the patient's disease was already advanced. However, there had been no previous examination. Among the 4 pregnancies that occurred while the mothers were undergoing treatment and the 18 after the disease apparently was well controlled, there was no evidence of change in the pulmonary condition.

Deaths from Tuberculosis

The group whose deaths occurred because of tuberculosis consists of 51 women who were first examined between 1922 and 1951. All except 4 nurses were housewives.

Thirty-seven patients gave no history of known contact with contagious cases of tuberculosis. The remaining 14 had previous contact as follows: 9 with tuberculous immediate family members, one in a home as a maid, and 4 as student nurses. All 51 had advanced disease, and tubercle bacilli were demonstrated.

On first examination, the patients ranged in age from 20 to 69, with an average of 36.8 years. At the time of death, the age range was from 20 to 89 years, with an average of 38. Twelve were 40 years or older on the first examination and 15 others were in that age bracket before death. These 51 women had 254 person-life years of follow-up study before death.

The patients had 106 children, 84 of whom were born before their mothers were first examined and 22 while the mothers were being treated. Four had one pregnancy each terminated in 1928, 1929, 1936, and 1941.

Questionable influence of pregnancy on tuberculosis: Eleven of the women went through pregnancy within one or two years of the time their disease was diagnosed.

At the age of 18 years, one woman married a widower with six children. A year later she had an apparently normal pregnancy and delivery. When she was 20 years old (February, 1935), she delivered a seven-month viable baby. About that time, her first chest examination revealed advanced disease in the left lung, from which she died in three months. There was no past record to determine the source of her infection or when clinical lesions evolved.

A woman of 17 years had an apparently normal pregnancy and delivery in 1925. Her next three children were born in 1926, 1928, and 1930. She stated that after the birth of her last baby, she experienced some fatigue and had a hacking cough. She was found to have pulmonary tuberculosis with sputum positive for *M. tuberculosis* in May, 1930. She was in a sanatorium for approximately a year and did well for three years thereafter. However, in 1934 she returned to the sanatorium with reactivation, but left against medical advice in 1937. She died at home in 1939.

A woman of 32 years went through normal pregnancy and delivery in 1940. Her second child was born in 1941. She stated that, just after the birth of the second baby, she began to cough and have sputum. Although her husband was a physician, her first examination in May, 1941, revealed advanced pulmonary tuberculosis. She was admitted to a sanatorium, but died in August, 1942.

A patient of 23 years stated that she developed hoarseness and cough after the birth of her first baby in November, 1927. Her first examination then revealed advanced pulmonary tuberculosis. She was admitted to a sanatorium in November, 1929, where she died in July, 1931.

A woman of 21 years gave birth to a son on March 8, 1934. In May, 1935, on the first examination, she was found to have extensive disease in her left lung. She was institutionalized in June, 1935, with far advanced pulmonary disease, tuberculous laryngitis, and enteritis. All treatment was unavailing. She left the sanatorium against medical advice on March 7, 1936, to be home with her small child. She died there on April 9, 1936.

When first examined on May 29, 1930, a woman of 20 years was found to have far advanced pulmonary tuberculosis. She had complained of fatigue since her first baby was born on December 26, 1929. Her disease progressed and she died in 1934.

One woman had pleurisy with effusion when she was 22 years old in 1922. One child was born in 1919 and the other in 1921. In May, 1923, she had bilateral pulmonary disease with bacilli in the sputum. She was admitted to a sanatorium in September, 1923, and discharged in March, 1924. She was readmitted on June 25 and died there in September, 1924.

The two children of another woman were born in 1931 and October, 1933. In August, 1933 at the age of 27 years, she was found to have tuberculosis with infectious sputum. She did well on treatment until May, 1938, when reactivation occurred and she was admitted to a hospital and died in February, 1941.

A woman of 35 years gave birth to her only child on December 1, 1925. In February, 1926, she had marked enlargement of cervical lymph nodes. When first examined in July, 1926, she had far advanced pulmonary disease involving the left lung with marked enlargement of cervical lymph nodes. She did not respond to treatment and died in March, 1927.

Questionable aggravation or reactivation of tuberculous lesions: One woman had been treated in a sanatorium for 15 months, beginning in 1938. On March 6, 1944, her only child was born. When she was re-examined on July 28, 1944, the disease had increased. She had been in another state for two years and had had no examination between 1942 and March, 1944, when the baby was born. Therefore, it is not known when the disease reactivated.

At the age of 22 years, a woman was first examined in 1934 because she had been in contact with her father and a brother who died from tuberculosis. She reacted to tuberculin, but a roentgenogram of her chest was clear. In February, 1937, a lesion was in evidence in the upper lobe of the left lung. While on treatment, a small lesion appeared in the apex of the right lung in June, 1938, and the lesion in the left lung had increased; but both responded to treatment. Her only child was born on November 17, 1938. She did well until 1942 when the lesion in the right lung spread. She was admitted to a sanatorium in November, 1942, where she died in August, 1943.

A nurse of 28 years was found to have a tuberculous lesion in the left lung in December, 1929. In January, 1931, she discontinued treatment against advice and was married. Her first baby was born in October, 1931, and the second in October, 1932. She did well until she had an attack of whooping cough in the summer of 1934. The cough did not entirely disappear and, in July, 1935, other symptoms developed and she was referred to a sanatorium but left against advice on the ninth day. She then had extensive disease in both lungs and died in her home in January, 1936.

In 1911, at the age of 22 years, a woman had large cervical lymph nodes which subsided, but returned two years later when they were removed and found to be tuberculous. One year later she had a pulmonary hemorrhage and was sent to a sanatorium where she remained for seven months. She apparently had remained well until examination revealed pulmonary disease on July 4, 1928. She stated that she had had a cough since her last baby was born in February, 1928. Her other two children were then six and seven years old. From then until August, 1930, she was admitted to three sanatoriums, and died in 1931.

Among the remainder of the 51 women who died from tuberculosis, the births occurred so long before diagnosis that there seemed little possibility that they had influenced the development or reactivation of the disease. The 22 mothers whose 19 babies were born while they were on treatment showed no change in the tuberculous condition during pregnancy or the puerperium.

Women Still Living

There are 316 women still living, the majority of whom gave their occupation as "housewife"; next were 56 nurses, 31 students, 23 clerical workers, 8 executives, and 6 teachers.

The 56 nurses as students or graduates gave histories of contact with tuberculous patients. Fifty-seven had contact with tuberculous members of the immediate family, and 19 with associates at work or close friends. The remainder gave no history of contact with tuberculous patients.

Eighty-five had minimal and 231 advanced

disease on the first examination. Tubercle bacilli were recovered from 252.

These 316 women ranged from 10 to 74 years old and the average age was 27.5 on first examination. At the termination of this study, the ages ranged from 24 to 85, with an average of 49.1 years. The subjects have been observed 7,205 person-life years. At the time of the first examination, 29 were 40 years or older and, at termination of the study, 219 others had entered that age bracket.

The 316 women had 732 children, 224 of whom were born before the mothers' disease was diagnosed, 63 during treatment, and 445 after the disease apparently was well controlled. Pregnancy was terminated once in each of 10 women, in 5 between 1932 and 1939 and in 5 between 1940 and 1949. Another patient had two pregnancies terminated, one in 1944 and one in 1945. These terminations had no apparent effect on the pulmonary lesions.

Questionable influence of pregnancy on development of pulmonary disease: Of the 316 women, 119 had gone through pregnancy prior to the diagnosis of tuberculosis. There were 25 who delivered babies within a year or less of the time of the diagnosis, and 11 others within two years.

A woman at the age of 25 years in 1921 had a normal pregnancy and delivery. However, she developed pleurisy with effusion a few days before her second baby was born in 1929. Her third pregnancy in 1930 was uneventful. In 1931 she was admitted to a sanatorium with far advanced tuberculosis. She made a good recovery and remained well until 1956, at the age of 60 years, when she developed generalized miliary tuberculosis and was treated successfully.

A woman of 25 years stated that after the birth of her only child in October, 1930, she experienced considerable fatigue and began to raise a small amount of sputum which was found to contain tubercle bacilli. She responded promptly to treatment and has remained in excellent health.

In the remainder of the 36 women whose babies were born within one or two years of the time the diagnosis of tuberculosis was made, there was nothing found to indicate that the course of tuberculosis had been altered by pregnancy or puerperium.

Among the 42 mothers who *delivered babies during their period of treatment,* one had pleurisy with effusion in April, 1945. A daughter was born in June, 1946, and her second child in April, 1949. In August of that year, a small area of disease appeared in the upper lobe of her left lung which was treated successfully.

A woman had a lesion in her left lung in 1947. One year later it had increased in size. She was in a sanatorium from September, 1949, to February, 1950. Her first baby was born in 1950, the second in 1951, and the third in 1954. She was readmitted to the sanatorium in January, 1955, and was discharged in February, 1956. The fourth baby was born in September, 1956. She has shown no evidence of reactivation of disease since leaving the sanatorium in February, 1956.

At the age of 21 in 1923, a student nurse was found to have pulmonary tuberculosis. She worked as a nurse after discharge from a sanatorium in 1924. In August, 1930, her first child was born with no apparent change in the lung condition. Three years later a baby was born prematurely and did not survive. In August, 1936, she delivered the third child. This was followed by insomnia, anxiety, and fatigue. She then was found to have a lesion in the opposite lung. In 1957 she wrote, "I think if I had rested more and been able to have adequate help after the birth of the second and third children, I could have avoided a break-down."

A woman of 20 years reacted to tuberculin, but the roentgenogram of her chest was clear in 1940. In the spring of 1943, a small area of disease was demonstrable in the upper lobe of the left lung. She was graduating from a university and was advised to keep this lesion under close medical observation. She was married and her first baby was born in April, 1947, and the second in March, 1950. She had no examination after graduation until January, 1953, when extensive bilateral disease was present. She responded to treatment and has remained well.

A Negro girl was first examined in August, 1934, when she was 12 years old. Clinical lesions were present in the upper lobe of each lung. She was admitted to a sanatorium, but left against medical advice in May, 1939. She was then married and her first child was born in 1945, the second in 1948, and the third in 1949. She was readmitted to the sanatorium in February, 1956, with marked spread of the disease in both lungs. She was discharged in June, 1957, and there has been no evidence of reactivation. Her fourth child was born in 1958, with no apparent ill effect.

A nurse of 32 years had an apparently normal pregnancy and delivery in 1938. Her second child was born in 1939, and the third in 1941. Her first examination in April, 1943, revealed advanced pulmonary tuberculosis. She refused sanatorium care until January, 1944, but left against advice in April of that year. She was readmitted in June, 1945, and had a three-stage thoracoplasty, but again left

against advice in July, 1946. In 1949, there was an increase of disease in the contralateral lung. She rejected advice to return to the sanatorium. A surgeon refused to attempt resectional surgery because of inadequate lung capacity. She was placed on antituberculous drugs in 1954, and her lesions are now apparently stable.

The remaining 155 women *delivered 453 babies after the mother's disease was apparently controlled.* In none of these women was there evidence that pregnancy and puerperium changed the status of the mother's residual lesions.

Thirty-six of these women have had 3 children, 21 have had 4, 7 have had 5, 4 have had 6, and 2 have had 8 children. The remaining 85 have had only one or two children.

One mother at the age of 17 in 1920 was hospitalized for pulmonary tuberculosis with tubercle bacilli in the sputum. Soon thereafter she was found to have tuberculosis of the spine for which she was treated in a sanatorium until June, 1923. Her 3 children were born by cesarean section in 1928, 1932, and 1934. There has been no evidence of aggravation or reactivation of disease in her chest or spine in the past 37 years.

In 1943, at the age of 25 years a woman was treated in a sanatorium for advanced pulmonary tuberculosis with sputum positive for *M. tuberculosis.* She suffered much mental anguish because she believed it would not be possible to have more than the one child who was born before her disease was diagnosed. However, she delivered normal children in 1952, 1953, and 1960, with no apparent change in her chest condition since 1945.

At the age of 14 years, a woman was admitted to a sanatorium with pulmonary tuberculosis and infectious sputum in 1938. She was in the sanatorium 20 months, followed by bed rest at home for two years. Her first child was born in August, 1941, and the remaining 4 in 1949, 1953, 1954, and 1956. No pregnancy had any apparent effect on her tuberculosis.

A student nurse had been a nonreactor to tuberculin until 1943 after duty on a tuberculosis service. Six months after graduation in 1944, she had had a demonstrable pulmonary lesion with tubercle bacilli in her sputum. She was on treatment for three years. Her first baby was born in 1947 and the next 5 were born in 1950, 1951, 1952, 1954, and 1956. There has been no apparent change in her chest findings since treatment was discontinued.

In 1934, a 19-year-old woman had cavitary pulmonary tuberculosis with tubercle bacilli in her sputum. She was institutionalized until 1937. Her babies were born in 1946, 1947, 1948, 1951, 1952, 1953, 1955, and 1957. No apparent change has appeared in her lung condition since 1937.

Children of Tuberculous Mothers

Among the *34 children of the 21 mothers who died from nontuberculous conditions,* 11 were not tested with tuberculin. Of the 23 whose tests were recorded, 17 did not become reactors. Of the 6 who reacted to tuberculin, 5 were born before the mother's disease was diagnosed and one became a reactor while the mother was still on treatment.

One mother whose first examination revealed chronic cavitary disease at the age of 55 in 1936 had lost a daughter from tuberculosis in 1928. This was the only case of demonstrable lesions among the 34 children of the 21 mothers in this group.

Among the *106 children of the 51 women who died from tuberculosis,* 84 were born before there was a diagnosis of tuberculosis, and 22 during treatment.

Sixty-two were tested with tuberculin and 53 reacted. Fifty-one were born before their mothers' disease was diagnosed and 11 during treatment.

Six children in this group presented demonstrable primary pulmonary infiltrates. None had clinical tuberculosis.

Clinical lesions: Three children developed acute clinical tuberculosis. One mother, 35 years old in 1926 when her disease was first diagnosed, had given birth to her only child in December, 1925. This child died at the age of seven months from tuberculous meningitis. Another, who was found to have pulmonary tuberculosis at the age of 24 years in 1929, lost her only child from tuberculous meningitis at the age of four years in 1933. The third, a mother of 32 years in 1954, had her third child in December, 1953. This child had miliary tuberculosis at the age of nine months, but was treated successfully by antituberculous drugs.

A woman with infectious disease at the age of 21 years gave birth to a son in March, 1934. When he was three years old, tuberculosis was found involving the bodies of two lumbar vertebrae. He was admitted to a hospital for crippled children in January, 1938, and was discharged with apparent good result in May, 1941. Thereafter, he was readmitted to the same hospital five times, the last admittance being in January, 1947, and he died there on July 19, 1947,

from amyloidosis and uremia secondary to tuberculosis of the lumbar spine.

A mother who was 47 years old in 1936 when her disease appeared had one son who developed chronic clinical pulmonary tuberculosis in 1934 at the age of 26 years. When the son's disease was in evidence in 1934, the roentgenogram of the mother's chest was clear.

A mother who was 58 years old in 1939 when her disease was first found lost one son from tuberculosis in 1937. A daughter of 25 years had clinical pulmonary disease in 1939 and another daughter had clinical disease at the age of 35 in 1952.

Before first examination here, the following three mothers had lost a total of 5 daughters from tuberculosis. However, there is no information available concerning tuberculin reactions and when clinical lesions became manifest. A mother of 62 years when first examined in 1931 had a daughter who died from tuberculosis in 1927. The daughter of a woman of 63 years, first examined in 1928, had died from tuberculosis at the age of 11 years in 1915, and another daughter at the age of 31 in 1919. A mother who was 69 years old in 1930 had lost a daughter at the age of 19 years in 1921, and another 36 years old in 1924.

Of the *732 children of the 316 mothers still living*, 662 were tested with tuberculin, 107 (16.2 per cent) of whom reacted. Of the 107 reactors, 75 (70 per cent) were among the 224 born before the mother's disease was diagnosed, 10 (9.3 per cent) were among the 63 born during, and 22 (20.6 per cent) among the 445 after, treatment.

The preponderance of reactors among those born before diagnosis was due in part to contagious tuberculosis existing before the mothers were aware of its presence. For example, a daughter born before her mother's disease was discovered reacted to tuberculin while 2 children born after the mother's disease was treated are nonreactors.

Among the 224 born before the mothers' disease was diagnosed, 75 (33.5 per cent) reacted to tuberculin. Among the 63 children born while their mothers were undergoing treatment, 10 (15.9 per cent) became reactors. Such infections were sometimes due to mothers refusing to leave the home, departing from the sanatorium against medical advice, or to later reactivation of disease. For example, one woman who had 4 small children left the sanatorium against medical advice, whereupon each child became a reactor to tuberculin. Of the 445 children born after their mothers had been treated, only 22 (4.9 per cent) became reactors to tuberculin.

Fourteen (1.9 per cent) of the 732 children of 316 mothers received BCG. Most of the mothers were in a sanatorium when BCG was administered. In one case, it was given on a daughter's entrance to a school of nursing.

Among the 107 children who became reactors to tuberculin, 9 (8.4 per cent) had *demonstrable primary pulmonary infiltrates*. Four of the children remained in the home while 5 were institutionalized, one of whom had a lesion resected. None had clinical manifestations and the eight unresected infiltrates resolved in the usual manner.

Clinical disease:

A woman was first examined on October 24, 1929, because her infant had recently died from tuberculous meningitis. The mother had contagious pulmonary tuberculosis.

A nurse of 37 years in 1943 was first examined because a daughter born in 1939 had recently died from tuberculous meningitis. The mother had far advanced contagious tuberculosis. Another daughter born in May, 1942, died in November, 1943, from miliary tuberculosis and meningitis.

A 23-year-old woman in 1907 had axillary lymph nodes which required lancing. She had pleurisy in 1920. On examination here in October, 1927, she had advanced contagious pulmonary tuberculosis. In 1929, a daughter of 17 years died from pulmonary tuberculosis.

A 23-year-old woman had contagious pulmonary tuberculosis for which she was admitted to a sanatorium in 1926. Her 10-year-old daughter reacted to tuberculin. In 1934, this daughter had advanced bilateral pulmonary tuberculosis which was treated successfully.

In 1926, at the age of 20 years, a woman was admitted to a sanatorium with pulmonary tuberculosis. She remained only nine months and was in bed at home for one year. A daughter was born in 1934 and a son in 1939. In 1945 the mother had extensive cavitary pulmonary disease. She refused hospitalization. The daughter born in 1934 became a tuberculin reactor, but did not develop clinical pulmonary disease with sputum positive for tubercle bacilli until 1953.

On first examination in November, 1929, at the age of 40 years, a woman had advanced pulmonary tuberculosis. In December, 1934, her daughter, then

25 years old, reported with pulmonary involvement and sputum positive for tubercle bacilli.

A teacher, on first examination in August, 1936, at the age of 37 years, had contagious pulmonary tuberculosis. Her only child was three years old. Treatment was administered, but she discontinued against advice in April, 1938. Her daughter at the age of 15 years in December, 1948, had pulmonary tuberculosis which required six and one-half years of treatment.

Discussion

Although tubercle bacilli were not recovered in 68 of the 388 women, they had diagnoses of clinical tuberculosis based largely on the tuberculin reaction, presence of roentgenographic shadow-casting lesions, and course of disease.

In the 1920's and early 1930's, observations on sizeable groups of people revealed that each tuberculin reactor is a potential case of clinical tuberculosis. Periodic examination of a large number of adolescents and young adults whose only evidence of tuberculosis at the beginning was the tuberculin reaction disclosed that chronic clinical lesions which evolve can usually be detected by the roentgenographic shadows they cast before tubercle bacilli are recovered in gastric washings or sputum. Since some of these patients refused treatment, their lesions were observed to slowly extend until bacilli were recovered. Therefore, it was obvious that most cases of chronic pulmonary tuberculosis could be detected before they became contagious by periodic examination of adolescent and adult tuberculin reactors. Moreover, treatment at that time usually prevents the disease from becoming contagious (11–19). Some such cases are included among the 68 mothers in whom tubercle bacilli were not demonstrated.

Among the women who were not hospitalized were those who refused to leave home and those for whom it was not recommended because tubercle bacilli had not been demonstrated or the disease was readily brought under control by current methods of treatment which often also prevented tubercle bacilli from appearing in the sputum. In such cases, a well regulated life, artificial pneumothorax, and—later—antituberculous drugs (together with sufficient information about the disease) constituted the treatment.

A number of cases have been cited which, in the absence of information on the natural history of tuberculosis, might incriminate pregnancy as a cause of evolution of clinical disease or reactivation of that already present. The writer's observations have included a larger number of women with tuberculosis in the absence of pregnancy. Nothing has occurred among the women reported in this paper that has not been observed among unmarried and married women who have not undergone pregnancy. No evidence has accrued during the observations to indicate that pregnancy has had good or bad effect on tuberculosis. Nevertheless, many pathetic situations have been observed. For example, a teacher at the age of 26 in 1930 had a diagnosis of advanced contagious tuberculosis. She was hospitalized and restored to good general health. In 1937 there was slight reactivation which was soon overcome. However, she was advised by her family physician never to marry. She remained at home and took care of her father and mother who died in 1953 and 1957, respectively. She now resides alone in the family ten-room house without the pleasures and benefits of a family, and in her letter of July 19, 1959, stated that she has been in perfect health since 1938.

There is also a large number of tuberculous women who married against the recommendation of their physicians, but were strongly advised to avoid pregnancy. An example is one treated in 1931 and 1932 who wrote on June 10, 1960, "We have no children; several doctors advised against it, but sometimes I feel that they were too conservative as with all of the lifting and physical and mental strain of my mother's care, I surely could have taken pregnancy and childbirth in stride." To satisfy their desire, a considerable number of such mothers have adopted children.

It would appear that enough evidence has been produced, including that of other physicians from various places, to cast the belief that pregnancy and puerperium are deleterious to tuberculosis to the limbo of anachronisms.

For the greater part of the first three decades of these observations, tuberculosis was regarded as a legitimate reason for *termination of pregnancy*. However, among the 388 women traced, pregnancy was terminated in only 16 with a total of 21 terminations; one woman had five such terminations.

These 21 terminations were performed between 1927 and 1949. There was no evidence that the course of the patients' disease was changed by this procedure.

Tuberculosis in old age apparently has always

been a serious problem but, because of treatment, more people with this disease are entering the old age period beginning at 40 years, a considerable number of whom are destined to have reactivations which may occur at any time throughout the life span.

Among the 404 women included in this study, 46 were already 40 or older when first seen here. Moreover, among those who died from nontuberculous conditions and those from tuberculosis, 42 were in this upper age bracket at the time of death, and among those still living 248 are 40 years or older. Thus, 290 reached the upper age bracket, 248 of whom are still living.

Some of the reasons not all children of the tuberculous mothers were tested are: Some had attained adulthood and were living elsewhere before their mothers' disease was detected. Two mothers refused information concerning the testing of their total of eight children. One mother's four children were not tested because of expense. She was waiting for the test to be administered gratis when they started school. Other mothers with three and four children made similar statements, some believing children should not be tested before starting school. A mother wrote that their family physician does not stock tuberculin, but he would order it. One said the family physician had advised her not to use the tuberculin test because most likely her children were positive and, therefore, they should just have roentgenograms annually after the age of about three years. Fourteen children received BCG elsewhere and were not tested in this study.

While some of these omissions, particularly among the older women, occurred before the importance of the tuberculin test was generally accepted, a larger number happened more recently. Thus, there is need for additional extensive and intensive educational work directed by tuberculosis associations.

Not all children of tuberculous mothers who become invaded acquire their infections from their mothers. One mother who had infected her 10-year-old daughter about 1926, but made good recovery, had reactivation of her disease after moving into the home of this daughter in 1953, and she infected a grandchild. In 4 cases, the children developed infections and became reactors during exposure to their contagious fathers before their mothers had clinical disease. One mother stated that her son became a reactor while in medical school; another that her daughter did not react until she was nearly through nursing school. One reported that her son became a reactor while in the Navy. Therefore, no matter how well the mother's disease appears to be controlled, the family should be alerted to other possible sources of infection.

The necessity of educational work is emphasized by the fact that among the 388 women studied or traced, 133 (34.3 per cent) had no information as to when or where they came in contact with contagious cases of tuberculosis. Downes (20) discovered that approximately two-thirds of the patients who developed tuberculosis in a community under her observation had contracted it from sources outside the family. It is a reflection on any community to fail to know the location of contagious cases and even the location of the tuberculin reactors. Inasmuch as many contagious cases are unsuspected, even by the patients themselves, there is little likelihood that this problem will be entirely solved until universal periodic tuberculin testing is adopted.

Among 872 children of the 388 mothers traced, 747 (85.6 per cent) were tested with tuberculin; 166 reacted. Among the 62 children of mothers who died from tuberculosis, 53 of those tested (85.5 per cent) reacted. This is understandable because mothers, if hospitalized, frequently returned to their homes before death. Indeed, during the first decade of this study it was not unusual for sanatorium staffs to recommend that hopeless cases "return to their homes to die."

Of the 662 children tested of mothers still living, 107 (16.2 per cent) reacted. However, 87 (81.3 per cent) of the reactors were born before the mother's disease was diagnosed or during her treatment. Among the women still living, 445 children were born after the disease was apparently controlled; 22 (4.9 per cent) became infected. This percentage is only slightly higher than that of present grade school children in Minneapolis (21).

Since the pathologic work of Ghon (22), it has been recognized that tuberculous lesions of at least the primary type exist in the bodies of all persons who react to tuberculin due to tubercle bacilli. However, the majority do not attain size, consistency, or have a location possible of demonstration by present methods of ex-

amination. There seems no doubt that such lesions were present in the 166 children who became tuberculin reactors in this study, but in only 15 (9.1 per cent) was it possible to locate the lesions. This is a higher percentage than usual since 4 of the 5 children of one mother had demonstrable pulmonary infiltrates simultaneously on first examination.

In the early 1920's, demonstrable primary pulmonary infiltrates, as observed in both children and young adults, were not understood and were considered exceedingly serious clinical conditions. However, observations over the decades revealed that such infiltrates per se take the same benign course regardless of how much or how little treatment is administered (14). Several of the 15 children with demonstrable primary pulmonary infiltrates in this study had sanatorium treatment. Others remained in their homes with no treatment. One had an infiltrate resected under the protection of antituberculous drugs. The end result was the same in all 15, namely, no illness. To date, no clinical lesion has appeared.

Acute forms of tuberculosis developed in 6 of the 166 children who were tuberculin reactors. In 2 of them it was the meningitis which lead to finding contagious disease in their mothers. Four others had meningitis after their mothers were known to be tuberculous. Five of these children died prior to 1944. The sixth had miliary disease which occurred in 1954 and was treated successfully with antituberculous drugs.

Among the 166 tuberculin reactor children, 11 developed *chronic* tuberculosis; 4 of these died —3 from pulmonary disease (ages 17, 25, and 25) and one from tuberculosis of the spine (age 13 years). The remaining 7 ranged in age from 12 to 35 years when their pulmonary lesions were first diagnosed. All 7 recovered. Five were treated in the preantituberculous drug period.

Thus, among the 166 children, 6 have developed acute forms of tuberculosis from which 5 died. Eleven developed chronic forms (10 pulmonary and one spine), from which 4 died.

From the outset, one objective was to protect children from invasions of their mothers' tubercle bacilli. It was obvious that, unless this could be done effectively, tubercle bacilli would continue to perpetuate themselves as they had done for centuries. Therefore, it was a matter of informing mothers and affording every protective measure possible for their children.

A considerable number of the women had been placed on guard because of known exposure to tuberculous family members or other associates. Some had been exposed to contagion as students or graduate nurses. Others without known exposure had been found to react to tuberculin, et cetera. Therefore, they were being examined periodically for appearance of clinical disease. In this community, an extensive tuberculosis eradication campaign among university students, including students of nursing and medicine, began in 1927, and examination of hospital admissions was instituted in 1933. An extensive campaign was launched in the public and parochial schools in 1921 which provided for examination of contacts of children found to react to tuberculin. Thus, many persons had demonstrable tuberculosis detected in the silent stage.

Moreover, health departments provided splendid nursing and epidemiologic services which promptly brought important information to patients and families. National, state, and local tuberculosis associations constantly disseminated information throughout the area.

Sanatorium beds were increased in number so that prompt admission when indicated became possible. A practice was developed in sanatoriums and hospitals whereby babies had no contact with tuberculous mothers after birth for as long as the mother's disease was considered contagious. In 1930, the City Commissioner of Health requested physicians to report all tuberculin reactors to his department. In 1941, the Minnesota Trudeau Society voted unanimously to recommend that all physicians in the state report tuberculin reactors to their Boards of Health.

When the present observations began, the main treatment in vogue consisted of bed rest and pulmonary collapse, as indicated. In 1918, Slater (23) employed artificial pneumothorax for a pregnant woman, with excellent result. This has been used extensively in the present cases to great advantage as it so often converts sputum and controls lesions. During the last 10 years of this study, antituberculous drugs and resectional surgery have played a significant role.

The first woman in this group to receive streptomycin in 1945 had advanced disease involving both lungs, and her three children reacted to tuberculin. Streptomycin was not yet stocked by the sanatorium; therefore, it had to be purchased by her husband at considerable cost.

Since then 60 of these 316 women have received antituberculous drugs and 22 have had resectional surgery.

It seemed like a considerable achievement when the writer's office secretary who had a three-stage thoracoplasty in 1922 was married in 1929 and went through pregnancy and puerperium without incident at the age of 33 years in 1929; moreover, her daughter has remained uninfected for the 31 years of her life. For a mother to have more than one child after thoracoplasty was considered such an accomplishment that the case of a woman was reported (24) who had a three-stage thoracoplasty in 1922 and 1923, but went through normal pregnancy and delivery in 1929 and again in 1932. The two sons are still nonreactors to tuberculin. Moreover, as time passed, delivery of normal babies by women who had had thoracoplasties became almost commonplace. Normal pregnancies and deliveries with no apparent effect on tuberculous women following resections of segments, lobes, and lungs are no longer unusual.

There is no way of knowing in advance whether a mother's apparently well-controlled lesion will reactivate or new ones will appear. Therefore, periodic examinations, always including bacteriologic studies, are essential. An example is one of the mothers in this study, still living, who at the age of 18 years had contagious pulmonary tuberculosis. She was treated in a sanatorium for 17 months when the disease was classified "arrested." She reared 6 children, the oldest of whom is now nearly 40 years. Periodic examinations since discharge from the sanatorium revealed the lesion apparently well stabilized until 1953 when she was 53 years old; at that time tubercle bacilli were recovered. Apparently the reactivation was recent since she had been having frequent periodic examinations as a food handler. Moreover, throughout the years none of her 6 children reacted to tuberculin. Kendig and Rodgers (25) reported that of thirty infants of mothers with supposedly inactive tuberculosis, nine became infected. Thus, the greatest of care must be exercised with frequent bacteriologic studies to be certain that apparently well controlled lesions are detected promptly on reactivation. It is not unusual to recover tubercle bacilli when roentgenographic shadows appear unchanged.

It has been most gratifying to know that a preponderance of the women in this study have continued to have periodic examinations throughout the years, usually at yearly intervals and some semiannually. Moreover, most of them have tenaciously followed the policy of having their children tested annually or more often with tuberculin, and those who at any time have been found to react have thereafter had periodic roentgenographic examinations of the chest with complete examinations as indicated. In a considerable number, this is being promoted among their grandchildren.

Summary

The present report includes observations on 404 tuberculous women and their children. The mothers were first examined between 1920 and 1955. Sixteen have not been recently located. Of the remaining 388 mothers (96 per cent) who have been contacted or traced, 21 have died from nontuberculous conditions, and 51 from tuberculosis. Only 255 (65.7 per cent) of the 388 women who were traced or contacted were aware of having been associated with tuberculous persons or animals. Sixty-seven knew of immediate family contacts, 65 were nurses who had been exposed to tuberculous patients, and the remainder knew of contact with tuberculous schoolmates, working associates, et cetera. With proper use of the tuberculin test, the remaining 133 women could have been discovered before clinical disease developed and when many of the persons responsible for their infections could have been located.

Forty-six of the 404 women were forty years or older on first examination. Of the 72 who died, 42 had entered this age period and, of the 316 living, 248 are in this upper age bracket. The women who died from nontuberculous conditions had 454 person-life years of observation, those whose deaths were due to tuberculosis had 254, and those still living had 7,205 person-life years of observation.

The 388 women had 872 children, and 21 pregnancies were terminated because of tuberculosis. No evidence accrued to indicate that pregnancy has either a beneficial or a deleterious effect on tuberculosis. Of the 872 children, 747 (85.7 per cent) were tuberculin tested, and 166 (22.2 per cent) were reactors. Sixty-two children whose mothers died from tuberculosis were tested, and 53 (85.5 per cent) reacted. The majority of these children had intimate contact with their mothers before the disease was diag-

nosed or during its terminal stage. Fifteen (9.1 per cent) of the 166 children who developed primary tuberculosis had primary pulmonary infiltrates so located, of such size and consistency, as to be demonstrable by their roentgenographic shadows. None became clinical cases.

Seventeen (10.2 per cent) of the 166 tuberculin reactors have developed clinical disease. Six were cases of meningitis and miliary disease in young children. The one patient whose disease occurred in the antituberculous-drug era recovered. Eleven patients developed clinical tuberculosis—one had tuberculosis of the spine and 10 had pulmonary tuberculosis; 4 of the latter patients died prior to the era of antituberculous chemotherapy. The remaining 7 recovered, 5 of whom were ill during the pre-antituberculous-drug era.

The interval between invasion with tubercle bacilli and appearance of acute forms of tuberculosis, particularly meningitis and miliary disease, may range from only a few months to much longer periods of time. In chronic pulmonary disease, the interval is usually from more than one to many years. Thus, for persons who react to tuberculin, the importance of close surveillance is obvious. The natural history of transmission of tubercle bacilli by mothers to almost all of their children was observed in this study as it has been many times before. Although the natural history was markedly interrupted (581—77.8 per cent—of the children escaped infection), all of the infections could have been prevented if the available protective procedures, even in the first decade of this study, had been employed.

Sumario

La Historia Natural de la Tuberculosis en el Cuerpo Humano. III. Las Mujeres Tuberculosas y Sus Hijos

Esta comunicación comprende las observaciones realizadas en 404 mujeres tuberculosas y los hijos de ellas. Las mujeres fueron examinadas por primera vez entre 1920 y 1955. Con 16 de ellas no se ha dado recientemente. De las 388 madres restantes (96 por ciento), que han sido interrogadas o localizadas, 21 han fallecido de dolencias no tuberculosas y 51 de tuberculosis. Sólo 255 (65.7 por ciento) de las 388 mujeres localizadas o entrevistadas se habían dado cuenta de haber estado en contacto con personas o animales tuberculosos. Sesenta y siete estaban enteradas de contactos familiares inmediatos, 65 eran enfermeras que habían estado expuestas a enfermos tuberculosos y las restantes sabían de contactos con condiscípulas, compañeras de trabajo, etc., que padecían de tuberculosis. Con el uso adecuado de la prueba de la tuberculina, hubiera podido descubrirse a las restantes 133 mujeres antes de presentarse la enfermedad clínica y cuando hubiera podido darse con muchas de las personas causantes de sus infecciones.

Cuarenta y seis de las 404 mujeres tenían cuarenta años o más en el primer examen. De las 72 fallecidas, 42 habían pasado a dicha edad, y de las 316 vivas, 248 se encuentran en esa casilla elevada de la vida. Las mujeres que fallecieron de dolencias no tuberculosas experimentaron 454 años de vida-persona de observación, teniendo aquéllas cuyas muertes se debieron a tuberculosis 254, y las todavía vivas, 7,205 años de vida-persona de observación.

Las 388 mujeres tuvieron 872 hijos, y se puso término a 21 embarazos debido a tuberculosis. No se obtuvieron datos indicativos de que la tuberculosis ejerza efecto ya beneficioso o nocivo sobre la tuberculosis. De los 872 niños, 747 (85.7 por ciento) fueron comprobados con tuberculina y 166 (22.2 por ciento) resultaron ser reactores. Sesenta y dos niños cuyas madres fallecieron de tuberculosis fueron comprobados y 53 (85.5 por ciento) reaccionaron. La mayoría de estos niños estuvo en íntimo contacto con las madres antes de diagnosticarse la enfermedad o durante el período terminal. Quince (9.1 por ciento) de los 166 niños que manifestaron tuberculosis primaria tenían infiltraciones pulmonares primarias localizadas de tal modo y de tales tamaño y consistencia que eran reconocibles por sus imágenes radiográficas. Ninguno de estos casos se volvió clínico.

Diecisiete (10.2 por ciento) de los 166 reactores a la tuberculina han manifestado enfermedad clínica. Seis fueron casos de meningitis y granulia en niños pequeños. El único enfermos cuya dolencia se presentó en la era de la medicación antituberculosa se repuso. Once enfermos manifestaron tuberculosis clínica—uno tenía tuberculosis raquídea y 10 tenían tuberculosis pulmonar; 4 de los últimos fallecieron antes de la era de la quimioterapia antituberculosa. Los 7 restantes se repusieron, habiéndose enfermado 5 antes de la era de la medicación antituberculosa.

El intervalo entre la invasión por bacilos tuberculosos y la aparición de formas agudas de tuberculosis, en particular meningitis y granulia, puede variar de no más de algunos meses a períodos mucho más prolongados de tiempo. En la afección pulmonar crónica, el intervalo suele variar de más de un año a muchos años. Por eso, para las personas que reaccionan a la tuberculina, es manifiesta la importancia de una vigilancia concienzuda. En

este estudio se ha observado, como muchas veces antes, la historia natural de la transmisión de bacilos tuberculosos por las madres a casi todos sus hijos. Aunque la historia natural tuvo notables interrupciones (581—77.8 por ciento—de los niños eludieron la infección), hubieran podido impedirse todas las infecciones de haberse empleado los procedimientos protectores disponibles, aun en el primer decenio de este estudio.

Resume

L'histoire naturelle de la tuberculose dans le corps humain. III. Femmes tuberculeuses et leurs enfants

Ce rapport comprend les observations de 404 femmes tuberculeuses et leurs enfants. Les mères furent examinées les premières entre 1920 et 1955. Seize n'ont pas été récemment trouvées. Parmi les 388 restant (96%) qui ont été contactées ou suivies, 21 sont mortes de maladies non tuberculeuses et 51 de tuberculose. Seulement 255 (65,7 per cent) des 388 femmes qui furent suivies ou contactées savaient qu'elles avaient fréquenté des personnes ou des animaux tuberculeux. Soixante-sept étaient au courant de contacts familiaux directs, 65 étaient des infirmières qui avaient été exposées à des malades tuberculeux, et le reste savait qu'il y avait eu des contacts avec des compagnons d'école tuberculeux, des compagnons de travail, etc. Avec un usage convenable du test à la tuberculine, le reste des 133 femmes aurait pû être découvert avant que la maladie ne se développa cliniquement et au moment où de nombreuses personnes responsables de leur infection auraient pû être retrouvées.

Quarante-six des 404 femmes avaient quarante ans ou plus au premier examen. Parmi les 72 qui moururent, 42 étaient dans cette limite d'âge, et parmi les 316 en vie, 248 sont dans la limite d'âge supérieure. Les femmes qui moururent de maladies non tuberculeuses eurent 254, et celles encore en vie eurent 7,205 années d'observation de vie.

Les 388 femmes eurent 872 enfants, et 21 grossesses se terminèrent à cause de la tuberculose. Il n'y eut pas d'évidence indiquant que la grossesse eut un effet soit avantageux soit nocif sur la tuberculose. Parmi les 872 enfants, 747 (85,7%) furent testés avec de la tuberculine, et 166 (22,2%) réagirent. Soixante-deux enfants, dont les mères moururent de tuberculose, furent testés et 53 (85,5%) réagirent. La majorité de ces enfants eut un contact étroit avec leur mère avant que la maladie fut diagnostiquée ou à sa période terminale. Quinze (9,1%) des 166 enfants qui développèrent une tuberculose primaire eurent des infiltrats pulmonaires primaires situés de telle sorte, de telle taille et de telle importance qu'ils purent se manifester par des ombres radiologiques. Aucun ne devint un cas clinique.

Dix-sept (10,2%) des 166 sujets ayant réagi à la tuberculine développèrent une forme clinique. Six furent des cas de méningite et des formes miliaires chez des jeunes enfants. Le seul malade dont la maladie se produisit lors de la période des médicaments antituberculeux en guérit. Onze malades développèrent une tuberculose clinique—un eut une tuberculose de la colonne vertébrale et 10 eurent une tuberculose pulmonaire; 4 de ces derniers malades moururent avant l'ère de la chimiothérapie antituberculeuse. Les 7 restant guérirent, 5 d'entre eux furent malades pendant l'ère avant les médicaments antituberculeux.

L'intervalle entre l'envahissement par les bacilles tuberculeux et l'apparition des formes aiguës de tuberculose, en particulier des formes méningées et miliaires, peuvent se ranger de quelques mois seulement à des périodes beaucoup plus longues. Dans des formes pulmonaires chroniques, l'intervalle est habituellement de plus d'une année à plusieurs années. Par conséquent, pour des personnes qui réagissent à la tuberculine, l'importance d'une surveillance étroite est evidente. L'histoire naturelle de la transmission de bacilles tuberculeux par les mères à presque tous leurs enfants fut observée dans cette étude comme cela l'avait été fait bien des fois auparavant. Bien que l'histoire naturelle fut nettement interrompue (581—77,8% des enfants échappèrent à l'infection), toutes les infections auraient pû être prévenues si on avait appliqué les mesures de protection disponibles, même pendant la première décade de cette étude.

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Case Reports

OBJECTIVE FUNCTIONAL IMPROVEMENT BY DECORTICATION AFTER TWENTY YEARS OF ARTIFICIAL PNEUMOTHORAX FOR PULMONARY TUBERCULOSIS[1]

Report of a Case and Review of the Literature

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Introduction

Pulmonary decortication in the surgical management of tuberculosis is well established. In tuberculous empyema the lung can be expanded to fill the empyema space, the infection can be controlled, and the lung function conserved by this nonmutilating procedure (1–4). Early reports on the results observed after re-expansion of a chronically collapsed tuberculous lung suggested that little or no functional improvement was possible (1, 5–7). Recent reports have been more favorable (4, 8–10).

The present writers have recently treated a patient in whom artificial pneumothorax had been maintained for almost twenty years. Decortication was successful in re-expanding the lung. The significant objective improvement in both unilateral and total pulmonary function made this case of particular interest.

Case Report

J.R. (C.G.H. No. 133440), a 42-year-old Spanish-American male, was referred to the Colorado General Hospital on March 20, 1960, for possible thoracotomy. His history of tuberculosis dated back to 1938. At that time, moderately advanced tuberculosis in the right upper lung and sputum containing acid-fast bacilli were noted. Two years of bed rest in a hospital and then at home produced clinical improvement, and the patient was allowed to return to work in 1940. Shortly thereafter, however, relapse occurred and the patient entered another hospital, again infectious and with cavitation in the right apex.

On September 1, 1940, a therapeutic pneumothorax was induced, allowing complete collapse of the right lung. Reversal of infectiousness occurred promptly; the patient was discharged, and pneumothorax was continued by his private physician. Refills were continued at approximately six- to eight-week intervals. He remained clinically well and was able to work at sedentary jobs, although somewhat limited by dyspnea on exertion. Progressive decrease in exercise tolerance was noted over the five years preceding admission. In 1959 the pneumothorax became complicated by empyema. Sudden onset of malaise, fatigue, low-grade fever, and dull, aching, right-sided chest pain occurred. The patient was admitted to the Cragmor Sanatorium, Colorado Springs, Colorado, on March 8, 1960. Slightly bloody, purulent pleural fluid was aspirated from the right hemithorax; this was negative for acid-fast bacilli on smear but subsequently revealed *M. tuberculosis* on culture; cultures for nontuberculous pathogens were negative. Because of the empyema and the thick calcified peel apparent on the chest roentgenogram, the patient was referred to Colorado General Hospital for consideration of surgery.

Examination revealed a slightly thin man who was comfortable at rest. He was not febrile, and the vital signs were normal. The trachea was deviated to the right. Multiple needle marks were noted in the right axillary area, the site of the many pneumothorax punctures. The right chest was flattened and immobile; the percussion note was decreased; fremitus was absent; and breath sounds were markedly diminished over the entire right side. The second aortic sound was of greater intensity than the second pulmonic sound. Abnormal precordial lifts and murmurs were not noted. Clubbing was not present.

The chest roentgenograms showed marked opacification of the right hemithorax with some calcified pleura and a persistent pneumothorax (figure 1). Bronchoscopy revealed no stenosis or endobronchial inflammation; the right segmental orifices were compressed and crowded medially but patent. Bronchograms revealed good arborization and no bronchiectasis or bronchopleural fistula. Angiography (figure 2) showed all branches of the right pulmonary artery to be well visualized, but crowded together and of small caliber. Catheterization demonstrated normal pulmonary artery pressure (25 systolic, 15 diastolic in mm. of mercury, with a mean of 20) at rest. Oxygen saturation was 91.7 per cent[2] at rest and 92 per cent at exercise. The main pulmonary artery pressure was

[1] From the Colorado General Hospital and Department of Medicine, University of Colorado School of Medicine; and the Webb Institute for Medical Research, University of Colorado Medical Center, Denver 20, Colorado.

[2] Normal saturation at Denver altitude is 92 per cent.

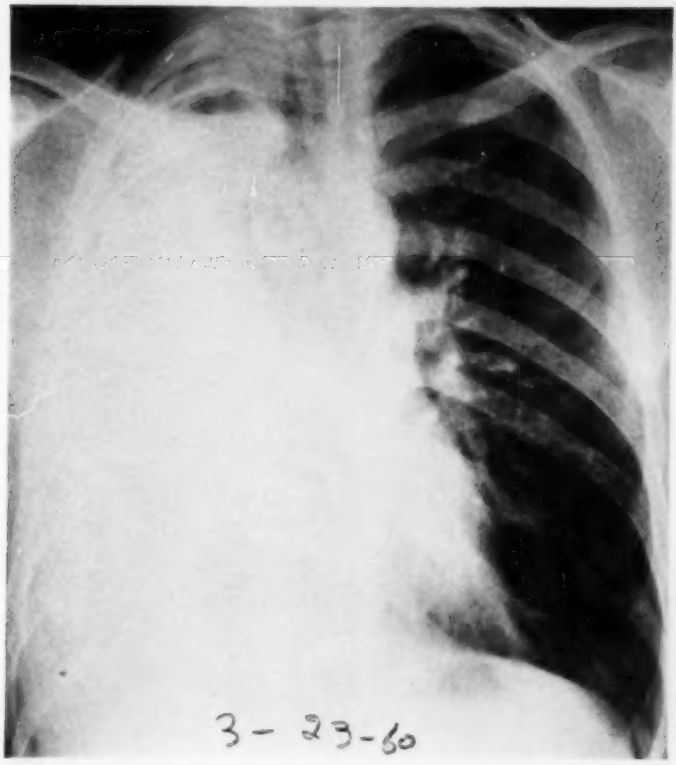

FIG. 1. Preoperative posterior-anterior chest roentgenogram shows marked opacification and some calcification, right.

moderately elevated to 50 systolic, 24 diastolic in mm. of mercury on exercise. Ventilatory tests demonstrated a moderate impairment with a vital capacity of 2,440 cc. (55 per cent of predicted normal) and a maximal breathing capacity of 47 liters per minute (31 per cent of predicted normal). Bronchospirometry demonstrated the defect to be markedly localized to the right; it should be noted that no gas exchange could be demonstrated preoperatively on the right (table 1). On April 3, 1960, two weeks after admission, the following chemotherapy regimen was started: isoniazid, 800 mg.; pyridoxine, 100 mg.; streptomycin, 1 gm. intramuscularly; and sodium PAS, 12 gm., each daily.

Decortication was performed on May 5, 1960.[3] The greatly thickened and partially calcified peel was removed with some difficulty. During the procedure the empyema cavity was entered, and approximately 3,000 ml. of serosanguineous fluid were removed. This proved to be sterile on culture for pyogens and negative for acid-fast bacilli on microscopy and culture. Immediately after the peel was removed, very satisfactory expansion of the lung was noted. No tuberculous foci were seen or palpated in the lung by the surgeons. The specimen was a gray and yellow peel of 1 to 2 cm. thickness surrounding a large empyema cavity which had a roughened lining. The wall was so calcified that it could scarcely be cut in places. Microscopic examination revealed broad sheets of hyalinized connective tissue containing nodules of lymphocytes and portions of old granulation tissue without necrosis. A fragment of pulmonary tissue contained collapsed alveoli, slight septal fibrosis, and thick-walled, small- and medium-sized arteries. No acid-fast bacilli were found in the resected pleura.

On the first postoperative day the chest roentgenogram showed partial expansion of the lung. The small air space filled first with fluid and then gradually re-expanded over the succeeding ten days. Recovery was uneventful. The patient was discharged on July 25, 1960, on continued therapy with isoniazid-PAS.

The patient returned on September 7, 1960, for follow-up studies. Marked improvement in the chest roentgenograms was evident (figure 3). He reported considerable improvement in exercise

[3] By Drs. R. K. Brown and P. Callaghan.

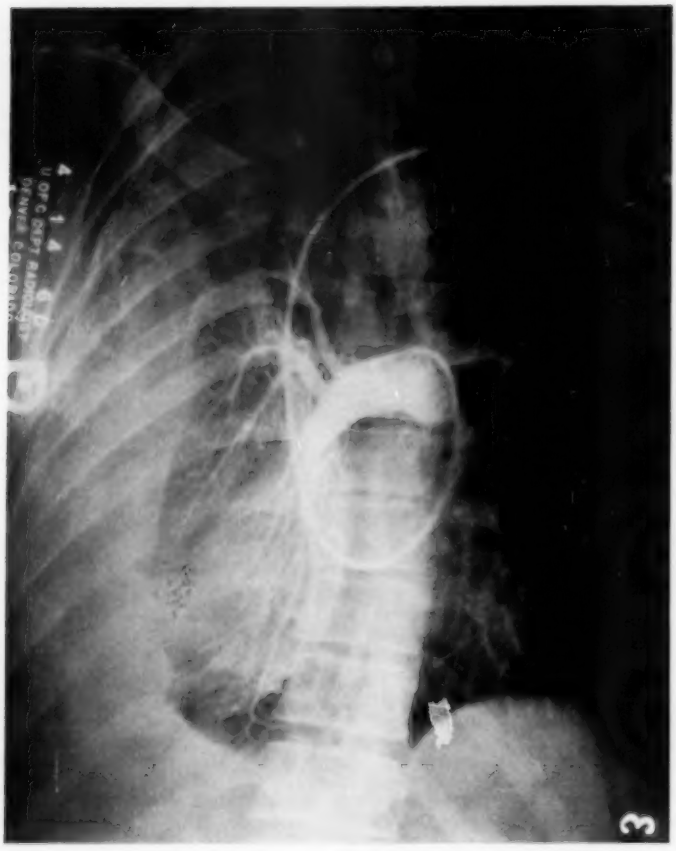

FIG. 2. Preoperative angiogram (32 ml. of 90 per cent diatrizoate given in the right antecubital vein) shows crowding but good patency and arborization, similar to a bronchogram.

tolerance. Dyspnea was present only on strenuous exertion. The results of the pulmonary function studies performed ten and eighteen weeks postoperatively are shown in table 2. After 18 weeks' post-decortication, the vital capacity was 3,380 cc., a 38 per cent improvement, and the maximal breathing capacity, 111 liters per minute, a 132 per cent improvement over the preoperative values. Bronchospirometry demonstrated participation of the right lung in both ventilation and gas exchange; the right lung contributed 37 per cent of the total ventilation, 22 per cent of the total oxygen consumption, and 30 per cent of the total carbon dioxide excretion.

DISCUSSION

In the case reported, decortication was successful in obliterating the infected pleural space and in improving pulmonary function. These two objectives were accomplished despite nearly twenty years of collapse of a lung and the recent empyema. It is impressive that a collapsed lung which has not functioned for twenty years could be re-expanded to fill its normal space and participate significantly in ventilation and respiration. This success is believed to have been possible because the collapsed lung had not become complicated by airway stenosis, bronchiectasis, or extensive pulmonary infiltration. Also, the pulmonary vasculature remained undamaged.

Numerous reports have appeared on the success of decortication in the treatment of empyema and in the re-expansion of a chronically

TABLE 1

PREOPERATIVE PULMONARY FUNCTION TESTS

Test	Observed	Predicted Normal
Vital capacity	2,440 cc.	4,430 cc.
F.E.V.$_1$*	1,360 cc. (55%)	3,765 cc. (85%)
MMF†	0.62 L. per sec.	4.37 ± 1.10 L. per sec.
MBC‡ (indirect)	47 L. per min.	150 L. per min.
Bronchospirometry	**Right Lung**	**Left Lung**
	per cent	*per cent*
Vital capacity	5	95
Oxygen uptake	0	100
CO_2 output	0	100

* Forced expiratory volume in one second
† Maximal midexpiratory flow
‡ Maximal breathing capacity

collapsed tuberculous lung (1, 5, 8–16). An historical review is beyond the scope of this paper, but attention is called to three articles which review most of the early publications on decortication (3, 13, 16). Experience with decortication during World War II in cases of fibrothorax following traumatic hemothorax showed that re-expansion of the lung and functional improvement were possible, even after long-standing collapse (5, 17–19).

In 1947 Gurd reported three cases of tuberculous empyema successfully corrected by decortication, and thus reopened interest in this procedure in tuberculosis (2). In 1948 Mulvehill first reported on decortication in postpneumothorax patients (9). It has subsequently been shown that, when decortication is undertaken early in tuberculosis and when the degree of parenchymal disease in the collapsed lung is

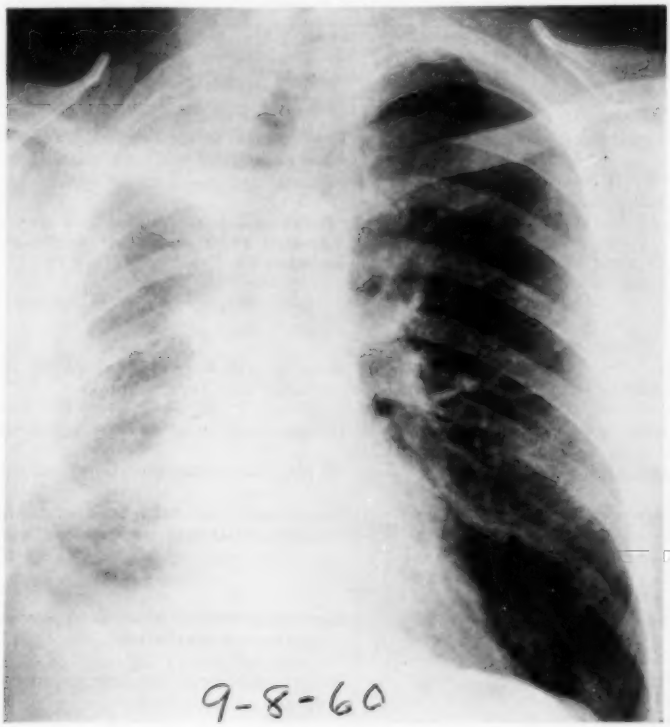

FIG. 3. Postoperative (18 weeks) posteroanterior chest roentgenogram shows good expansion of right lung.

TABLE 2

POSTOPERATIVE PULMONARY FUNCTION TESTS

Test	Ten Weeks after Decortication	Eighteen Weeks after Decortication
Vital capacity	3,160 cc.	3,380 cc.
$F.E.V._{.1}$	2,270 cc. (72%)	2,670 cc. (79%)
MMF	1.66 L. per sec.	2.60 L. per sec.
MBC	70 L. per min. (indirect)	111 L. per min. (direct)
Bronchospirometry 18 Weeks post-decortication	**Right Lung**	**Left Lung**
	per cent	*per cent*
Vital capacity	18	82
Ventilation	37	63
Oxygen uptake	22	78
CO_2 output	30	70

small, considerable return of function may be expected (4, 8, 10, 14, 19, 20). Patton and associates have shown that functional improvement may not be demonstrable until some months after decortication; he described a series studied at intervals after surgery (10). Elegant studies by Siebens give further testimony concerning the functional improvement following decortication (20). Reports of results after long-standing collapse in tuberculosis, however, have been few. Only 19 case reports could be found; these are summarized in table 3. Pulmonary function tests were performed on some of these patients, with conclusive objective evidence of improvement in only three cases (14, 20, 22). The present case and those reported previously (14, 20, 22) emphasize that prolonged collapse does not necessarily rule out improvement in lung function.

TABLE 3

NINETEEN REPORTED CASES OF DECORTICATION FOLLOWING COLLAPSE OF 10 YEARS OR LONGER

Author	Duration of Collapse before Decortication	Result
	years	
Dark (8)	10 12 24	Improvement in vital capacity and maximal breathing capacity
Ellis *et al.* (1)	12 12 16 16 16 17	"Functional improvement" in one case, one decreased function, others unchanged (details of studies not listed)
Falk *et al.* (22)	10	Bronchospirometry studies: modest increase in ventilation and oxygen uptake
Gordon and Welles (6)	26	Improvement in arterial oxygen saturation
Mulvehill and Klopstock (9)	14	No function studies reported; good expansion of lung
Samson *et al.* (4)	12 13 16 30	Improvement in total ventilation in one case; marked symptomatic improvement in all cases
Savage and Fleming (14)	20	Bronchospirometric studies: improvement in oxygen uptake and ventilation
Siebens *et al.* (20)	13	Bronchospirometric studies: improvement in oxygen uptake and ventilation
Weinberg *et al.* (15)	20	No function studies reported; poor expansion of lung

A recent bronchospirometric study stresses the importance of pleural disease in decreasing pulmonary function (23). Even minimal pleural thickening may cause considerable reduction in unilateral ventilation which is not compensated by an increase in function of the contralateral lung. The case reported clearly demonstrates that these losses may be reversible.

When the pathology of pulmonary collapse is considered, it is not surprising that return of function after re-expansion is possible. Although alveoli are collapsed and their walls approximated, the pulmonary architecture should not be obliterated by fibrosis unless the parenchyma also contain an inflammatory process (6). In some cases the postoperative increase in ventilation may be matched by improved perfusion of the corresponding capillaries (20). The present case shows the usual pattern, namely, a greater rise in ventilation volume than in oxygen uptake, indicating that perfusion has not kept pace with ventilation in the re-expanded lung.

Pneumothorax has now been replaced by chemotherapy and surgery, but patients with chronic collapse and imprisoned lung are still encountered. The favorable outcome in the present patient re-emphasizes the value of decortication in the present-day management of such cases.

Summary

A case is presented in which a tuberculous lung, collapsed for almost twenty years, was satisfactorily re-expanded by decortication. Improvement in both ventilation and perfusion of this lung has been well demonstrated by pre- and postoperative physiologic tests.

Sumario

Mejoría Funcional Objetiva por la Decorticación Tras Veinte Años de Neumotórax Terapéutico por Tuberculosis Pulmonar

Preséntase un caso, en el que un pulmón tuberculoso, aplastado durante casi veinte años, fué reexpandido satisfactoriamente por la decorticación. El mejoramiento tanto de la ventilación como de la perfusión de este pulmón ha quedado bien demostrado con pruebas fisiológicas pre- y postoperatorias.

Resume

Amélioration fonctionnelle objective par decortication après vingt ans de pneumothorax artificiel pour tuberculose pulmonaire

Les auteurs présentent un cas dans lequel un poumon tuberculeux, collabé pendant près de vingt ans, a été redilaté d'une façon satisfaisante par décortication. Une amélioration à la fois de la ventilation et de la circulation dans ce poumon a été bien prouvée par des tests physiologiques pré et post-opératoires.

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CAVITARY PULMONARY CRYPTOCOCCOSIS[1]

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Introduction

In his recent excellent review of cryptococcosis, Littman (1) points out the infrequency of cavitation in the pulmonary lesions of this disease. A report from The National Institutes of Health (2) indicates similarly that pulmonary cavitation seldom occurs. Thus, the present writer was motivated to report the following case.

Case Report

H.P., a 27-year-old army enlisted man on active duty was transferred to Fitzsimons General Hospital for admission on May 3, 1956.

Present illness: In March, 1956, at a military installation in Texas, the patient had a chest roentgenogram in connection with a regularly scheduled food-handler's examination. An abnormality was seen on the film and the patient was admitted for evaluation. He denied any pulmonary symptoms and knew of no contacts with tuberculosis. Skin testing revealed a positive reaction to second-strength tuberculin, a positive reaction to 1:100 histoplasmin, and a negative reaction to 1:100 coccidioidin. One gastric culture, taken shortly after admission, was reported as positive for *M. tuberculosis.*

Past and family history: The patient had lived in Pennsylvania until the time he entered the military service in 1946. During his military service he had been stationed in Louisiana, Kentucky, Texas, Europe, and Korea. While in Texas he had made trips into west Texas. There was no family history of tuberculosis.

Physical examination: Physical examination at the time of admission to Fitzsimons General Hospital was completely normal.

Laboratory data: Urinalysis and hemogram were normal. Skin testing substantiated the findings noted at the transferring hospital, as indicated above. Six gastric cultures were negative for acid-fast bacilli. Bronchoscopy was negative; gastric washings were not taken. Chest films taken shortly after the institution of antituberculous therapy (figure 1) and just before thoracotomy (figure 2), as well as a preoperative laminagram (figure 3), are shown.

Hospital course: On May 4, 1956, the patient started intramuscular streptomycin, 2 gm. twice weekly, and oral isoniazid, 300 mg. daily. He tolerated this treatment well; however, there was very little change in the appearance of the roentgenographic abnormality. Inasmuch as the one culture positive for *M. tuberculosis* was not corroborated and there was no roentgenographic response to five months of treatment, a right lower lobectomy was done on October 25, 1956. The patient made an uneventful postoperative recovery. Pathologic examination of the resected lobe (figure 4) showed a fibrocaseous granuloma, cavitary in nature, with *Cryptococcus neoformans* in the microscopic sections. Cultures of the specimen also revealed colonies of *C. neoformans.*

After a suitable postoperative convalescent period, the patient was returned to military duty.

Discussion

Localized pulmonary lesions attributable to *Cryptococcus neoformans,* in the absence of extrapulmonary infection or sputum cultures positive for *M. tuberculosis,* are usually diagnosed by microscopic and cultural examination of resected specimens obtained at the time of diagnostic thoracotomy and are seldom diagnosed preoperatively. This is likely due to the lack of a reliable skin-testing antigen and the relatively meager clinical experience with serologic studies (1) and is in contrast with the diagnostic studies readily available in certain other granulomatous diseases.

The absence of characteristic roentgenographic findings also contributes to the diagnostic difficulty in nondisseminated cryptococcosis. Recent papers (3, 4) would permit the changes to be grouped into three categories: (*a*) localized solitary masses usually without sharp, smooth borders; (*b*) infiltrative lesions, either single or multiple; and (*c*) multiple nodular densities.

In the nine cases of pulmonary involvement reported by Wolfe and Jacobson (3) two cases of cavitation were described, both of which occurred in patients with segmental types of infiltrates. Bonmati, Rogers, and Hopkins (4), in reporting seven cases, state that in some cases nodular lesions may undergo cavitation, although no open cavities were demonstrated in their series.

In the case herein presented, comment should be made on the report of one gastric culture

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FIG. 1. Posteroanterior chest film taken shortly after institution of antituberculous therapy. Note cavitary lesion in right lung field at level of eighth posterior intercostal space.

FIG. 2. Posteroanterior chest film taken preoperatively. Note lack of significant change after five months of therapy.

FIG. 3. Preoperative laminagram showing distinct cavity.

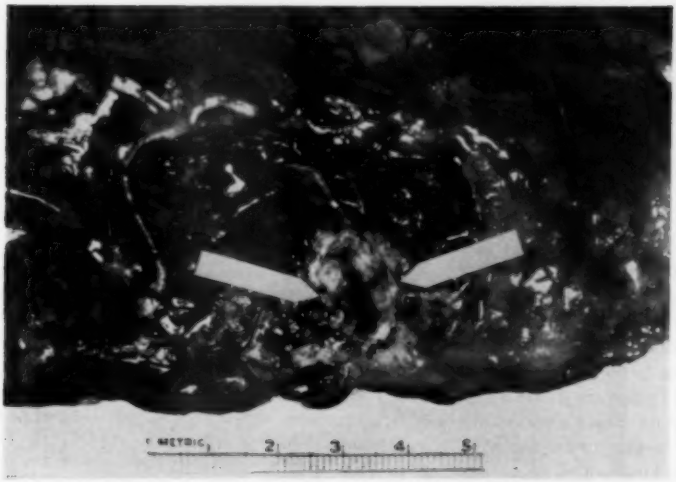

FIG. 4. Surgical specimen cut to show open cavitary lesion indicated by arrow.

positive for tubercle bacilli. This institution receives pulmonary patients from many scattered military and civilian medical facilities. It is not uncommon to have single, isolated, unconfirmed reports in patients subsequently found to have no evidence of active tuberculous infection. In this case, in view of the microscopic and cultural findings following thoracotomy, the reported gastric culture is therefore considered a false positive. Perhaps in this particular patient a more avid search for an etiologic agent would have resulted in a proper preoperative diagnosis. The lack of any significant roentgenographic response to antituberculous therapy cast doubt on the validity of the diagnosis of tuberculosis, and consequently thoracotomy was done.

SUMMARY

A case demonstrating the unusual finding of cavitation in a localized pulmonary cryptococcal lesion is reported. Lack of response to antituberculous therapy and inability to confirm a single reported gastric culture positive for tubercle bacilli led to thoracotomy. The etiologic diagnosis was made by laboratory examination of the resected lung specimen.

SUMARIO

Criptococosis Pulmonar Cavitaria

Se comunica un caso que revela el hallazgo extraño de cavernas en una lesión criptocócica. La falta de respuesta a la terapéutuca antituberculosa y la incapacidad para confirmar la obtención de un solo cultivo gástrico positivo para bacilos tuberculosos condujeron a la toracotomía. El diagnóstico etiológico se hizo por el examen en el laboratorio del ejemplar resecado del pulmón.

RESUME

Caverne pulmonaire dans la cryptococcose

L'auteur rapporte un cas montrant une découverte inhabituelle de caverne dans une lésion pulmonaire localisée cryptococcale. Le manque de réponse à la thérapeutique antituberculeuse et l'impossibilité de confirmer aucune culture gastrique positive pour les bacilles tuberculeux conduisit à une thoracotomie. Le diagnostic étiologique fut fait grace à l'examen de laboratoire de l'échantillon pulmonaire réséqué.

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BAGASSE DISEASE OF THE LUNGS[1]

Early Histopathologic Changes Demonstrated by Lung Biopsy

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(Received for publication March 13, 1961)

INTRODUCTION

Bagassosis is an industrial disease of increasing importance in areas where sugar cane is a major crop. Reports indicate that both repeated acute respiratory distress and permanent respiratory disability result from exposure to the dust from bales of dried bagasse. Sodeman and Pullen (1) described the histopathology in several patients with chronic disease. Exact knowledge is lacking of the initial changes which occur in the lungs of persons reacting to the unknown agent in this material. Such information would be of great value in guiding therapeutic programs. The following case, in which intercostal lung biopsy was performed with the use of a local anesthetic, illustrates the early histopathologic changes.

REPORT OF CASE

A 24-year-old white male was admitted to Ochsner Foundation Hospital on July 4, 1959, for evaluation of his illness. He had been employed for eleven months as a hammermill operator in a plant where paper is made from bagasse. The hammermill is the first machine through which the bagasse moves after the bales are broken open by men using axes in the same area of the plant.

The patient had lost about 10 pounds during the eight months preceding admission and had noted a tendency toward a dry, hacking cough in the afternoon. He smoked only occasionally. During the same period he had had what seemed to be typical tension headaches. Three weeks before admission, while at work, weakness and a "chilly feeling" developed, followed by increasing dyspnea and bilateral aching in the anterior portion of the chest. Coughing became more frequent. After work he consulted his physician, who found his temperature to be 103° F. and hospitalized him with a presumptive diagnosis of pneumonia, even though no evidence of consolidation could be seen in the roentgenogram of the chest. He remained in the hospital one week, during which time his temperature returned to normal, but cough and dyspnea on slight exertion persisted.

On admission to Ochsner Foundation Hospital, examination revealed no unusual physical abnormalities. On roentgenography of the chest, a fine granular and reticular pattern involving the lower two-thirds of each lung was noted (figure 1), but there was no prominence of the hilar structures or pleural involvement. The cardiac silhouette was normal. On fluoroscopy of the chest no evidence of air trapping was seen, and the results of electrocardiography were normal. The hemoglobin was 15.9 gm. per 100 ml. and the hematocrit was 49.5 per cent. The leukocyte count was 6,000 per cu. mm. with a differential count of 34 segmentals, 53 lymphocytes, and 13 monocytes. The Westergren sedimentation rate was 7 mm. per hour. Results of hepatic function studies were normal. No abnormalities were demonstrable in roentgenograms of the renal or gastrointestinal systems.

On July 8, 1959, with use of a local anesthetic, intercostal lung biopsy was performed through the fifth costal interspace on the left in the anterior axillary line. Microscopically, the sections showed pulmonary parenchyma with numerous, isolated, nodular lesions, composed of proliferating fibroblasts and foreign body giant cells as well as a lesser number of other inflammatory cells, most of which were macrophages and lymphocytes. The foreign body giant cells frequently contained a central foreign structure (figure 2), which changed or rotated the plane of polarization of light and appeared as a bright crystalline structure with a central opaque area corresponding to a central canal. These structures averaged 10 to 15 μ in diameter and had the general appearance of vegetable material or cellulose. Periodic-acid–Schiff and acid-fast stains were negative for any specific microorganisms. Beyond the nodular lesions, the pulmonary parenchyma was well preserved and the alevoli contained air. Some alveolar septa were slightly thickened, but others were thin and delicate. Enterococci and hemolytic streptococci were recovered from cultures of the pulmonary tissue, but no fungi or yeasts were seen. The patient was discharged with instructions to take prednisone, 15 mg. a day for one week, 10 mg. a day for two weeks, and 5 mg. a day for three weeks. He returned to work in the same plant one month after discharge from the hospital. He was assigned to work in another part of the plant and supposedly wore a respirator while at work.

Early in January, 1960, the patient reported that, one week previously, he had suffered a persistent dry cough, aching in the chest anteriorly, and a temperature of 103°F. for three days, with a sudden temperature fall during the week. Also, with exertion he exhibited the classic picture of hyperventilation with dizziness and numbness of

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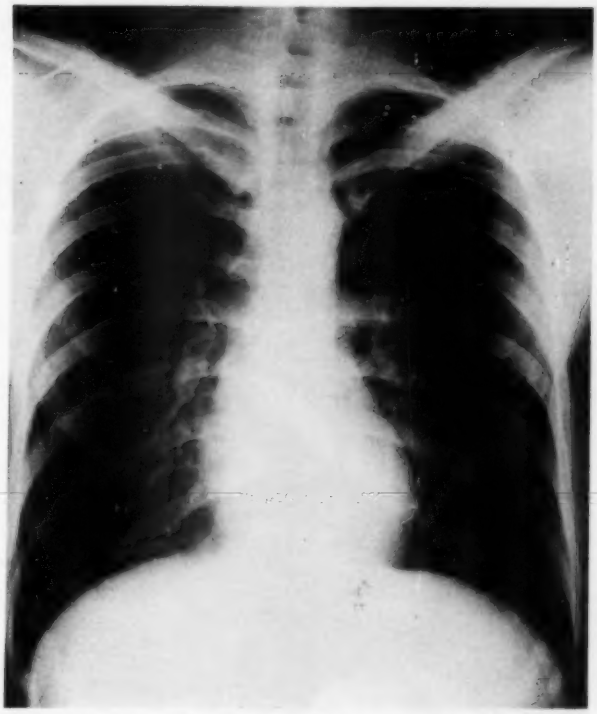

FIG. 1. Posteroanterior view of chest showing fine reticular infiltration in both lungs without hilar adenopathy.

the lips and fingertips. On roentgenography, accentuation of the reticular pattern of pulmonary markings in the bases was noted. This was thought perhaps to be actually a little less than on the previous examination.

The patient was readmitted on February 19, 1960, because of headaches thought to be functional. The electroencephalogram was reported as "borderline normal." On roentgenography the pathologic alterations detected previously had not progressed. The hemoglobin was 15.6 gm. per 100 ml.; hematocrit, 49 per cent; and leukocyte count, 7,500 per cu. mm. with 61 segmentals, 27 lymphocytes, 9 monocytes, 1 eosinophil, and 2 bands.

On February 24, 1960, results of complete pulmonary function studies indicated no impairment of ventilation or gas exchange. The oxygen tension was 84 mm. at rest and 88 mm. after exercise. The carbon dioxide tension was 32 mm. at rest and 25 mm. after exercise. The arterial blood pH remained at 7.41.

DISCUSSION

The microscopic observations described are compatible with the characteristic medical history. Patients complain of repeated brief episodes of fever, retrosternal discomfort, cough, occasionally blood-tinged sputum, malaise, and dyspnea. If contact is broken, the disease does not recur. Repeated exposures result in generalized pulmonary fibrosis and permanent pulmonary insufficiency of various degrees. These typical clinical manifestations have been described previously (2, 3). Evidence to date, including the striking clinical response to steroids in this case and as reported by Buechner and associates (3), would seem to indicate that this is a hypersensitivity reaction rather than the response to infection.

The earliest cases reported, both in this country and in England, resulted from the opening of bales of dried bagasse from Louisiana. Other cases have since been reported from such widely scattered areas as Italy (4), India, (5), Mexico (6), and Puerto Rico (7).

During 1953 and 1954 one of the present writers saw 12 patients with evidence of pulmonary disability resulting from repeated ex-

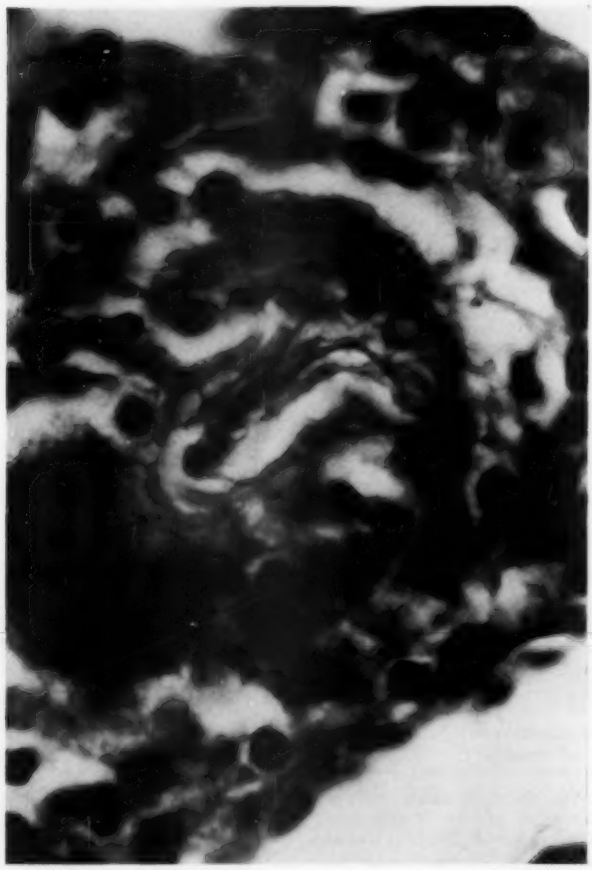

FIG. 2. High magnification of foreign-body type of giant cell in center of granuloma. Note the fragment of bagasse fiber in center of giant cell. (Hematoxylin and eosin stain, × 1,000)

posure to bagasse dust. These men were employed in a pilot plant designed to make newsprint from bagasse. Use of respirators seemed to have little effect in preventing attacks. Symptoms would occur in these persons when they were exposed to the dust from a distance of several hundred yards. When a decision was made to wet the bagasse bales before use, no further difficulty was reported in this plant. This was first demonstrated in England (8). In 1957, new management reverted to the use of dried bagasse initially, and 4 cases resulted. A plant using this same technique was opened in Puerto Rico in January, 1959. During the next year 69 cases of acute bagassosis were discovered, an incidence of almost 50 per cent of the total number of employees (7).

SUMMARY

A case of bagasse disease demonstrating the early histopathologic changes has been presented. This case indicates that steroid therapy is helpful in the management of early cases. Clinical evidence suggests that bagasse disease is a hypersensitivity reaction to an agent as yet unknown. Patients with the disease should not be exposed to bagasse dust. Wetting of bagasse before use will prevent development of the disease. Adequate respirators will not, entirely. Evidence is offered that this disease can be a

potentially crippling influence in the development of industries in some agricultural areas of the world where diversification of livelihood is needed badly.

Sumario

Bagazosis de los Pulmones: Incipientes Alteraciones Histopatológicas Reveladas por la Biopsia Pulmonar

Preséntase un caso de bagazosis que reveló las alteraciones histopatológicas incipientes. Indica este caso que la esteroidoterapia resulta útil en la asistencia de los casos incipientes. Los datos clínicos sugieren que la bagazosis es una reacción de hipersensibilidad a un agente todavía incógnito. Los enfermos no deben exponerse al polvo del bagazo. El humedecimiento de éste, antes de usarlo, impedirá la aparición de la enfermedad, mientras que el uso de respiradores adecuados no lo hará del todo. Se ofrecen pruebas de que esta dolencia puede constituir un factor potencialmente paralizador del desarrollo industrial en algunas regiones agrícolas en que necesitan con premura diversificación de los medios de subsistencia.

Resume

Bagassose des poumons: changements histopathologiques précoces montrés à la biopsie du poumon

Les auteurs présentent un cas de bagassose prouvant les changements histopathologiques précoces. Ce cas montre que la thérapeutique par les stéroïdes aide dans le traitement des cas pris tôt. L'évidence clinique fait penser que la bagassose est une réaction d'hypersensibilité à un agent encore inconnu. Des malades avec la maladie ne devraient pas être exposés à la poussière de bagasse. L'humidification de la bagasse avant de s'en servir assumera la prévention du développement de la maladie. Des respirateurs adéquats n'assureront pas une prévention complète. On a la preuve que cette maladie peut avoir une influence invalidante dans le développement d'industries dans certaines régions agricoles du monde où on a fortement besoin d'un changement de vie.

Acknowledgment

The help of Dr. G. M. Carrera, head of the Department of Pathology, Ochsner Clinic, in interpretation of the histopathologic picture and photomicrographs is gratefully acknowledged.

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Notes

COMPARISON OF CHARCOAL AGAR AND PETRAGNANI'S MEDIUM FOR THE ISOLATION OF MYCOBACTERIUM TUBERCULOSIS

Two disadvantages of the culture method as a diagnostic procedure in tuberculosis are the length of time required for growth to appear and the frequently unsatisfactory results due to contamination. In 1955, Whalen and Mallmann[1] described a charcoal agar medium and reported that it yielded a more rapid growth of *Mycobacterium tuberculosis* than Petragnani medium and had the added advantage of inhibiting contaminating organisms, particularly molds. It seemed important to evaluate these results in another laboratory.

In a preliminary study, 30 sputum specimens which were positive by stained slide examination were cultured on charcoal agar, on Petragnani medium, and on Löwenstein medium. The results with the new medium were encouraging, and in August, 1957, the study was extended to include all specimens submitted for culture and guinea pig inoculation. The use of Löwenstein medium was discontinued, and the comparison was restricted to Petragnani and charcoal agar media. The results with 3,567 specimens form the basis for this report, which does not confirm the usefulness of charcoal agar.

The media were prepared in the Michigan Department of Health Laboratory in Lansing under the direction of Dr. Bernice Graham.

The formula for the charcoal agar medium as given by Whalen and Mallmann is as follows:

Proteose-peptone #3 (Difco)	2.0 gm.
Disodium phosphate, Na_2HPO_4	2.5 gm.
Monopotassium phosphate, KH_2PO_4	1.0 gm.
Copper sulfate solution, $CuSO_4 \cdot {}_5H_2O$, 10 mg. per 100 ml.	1.0 ml.
Cobalt oxide solution, Co_2O_3, 10 mg. per 100 ml.	1.0 ml.
Ferric ammonium citrate	0.1 gm.
Dextrose	7.5 gm.
Glycerol	2.5 gm.
Savita® [2]	2.0 gm.
Triton A-20®	0.5 gm.
Activated charcoal (Nuchar C-190-N®)	4.0 gm.
Agar	15.6 gm.
Ethyl violet 6B, 0.1 per cent solution	2.5 ml.
Cycloheximide (Actidione®)	100 mg.
Penicillin	100,000 units
Distilled water, q.s.	1,000 ml.

All of the ingredients except the charcoal, ethyl violet, actidione, and penicillin were added to the water and boiled until the agar was dissolved. The reaction was adjusted to pH 6.8 to 7.0, and the volume restored with water. The ethyl violet and charcoal were added and mixed well. The medium was autoclaved for fifteen minutes at 121°C., and cooled to about 60°C. The cycloheximide (10 ml. of a 1 per cent aqueous solution) and the penicillin (10 ml. containing 100,000 units) were added aseptically. The medium was dispensed in screw-capped tubes and slanted.

The specimens were received from tuberculosis sanatoriums and from private physicians. About half were sputum specimens; a fourth, gastric washings; and the rest were urine, pleural fluid, and cerebrospinal fluid specimens. Each specimen was mixed with Hank's solution[2] (equal volumes) and allowed to stand in the 35°C. incubator for one hour, with occasional shaking by hand. The treated specimen was then neutralized with hydrochloric acid and centrifuged. The sediment was used to inoculate two tubes each of the media under study.

The inoculated slants were incubated at 35°C. and were examined at intervals for growth. Colonies resembling those of *M. tuberculosis* were studied by stained slide preparations.

The findings with respect to *M. tuberculosis* on charcoal agar and Petragnani medium from 3,567 specimens are given in table 1.

Eighty-one specimens yielded colonies typical of *M. tuberculosis* on both media. Seven were positive on charcoal agar but not on Petragnani medium, while 116 were positive on Petragnani and negative on the charcoal agar. In two instances *M. tuberculosis* was found on charcoal agar while the Petragnani medium was overgrown with contaminating organisms. Positive results were obtained in 90 specimens on the charcoal agar, representing 2.5 per cent of the total. In comparison, *M. tuberculosis* was found in 197 specimens, or 5.5 per cent, on the Petragnani medium.

The results obtained with the 206 specimens which yielded growth of *M. tuberculosis* on either or both media are given in table 2.

Growth was first observed in 36 specimens during the same week on both media. In two specimens, growth appeared earlier on the charcoal

[1] Whalen, J. W., and Mallmann, W. L.: Amer Rev Tuberc, 1955, *71*, 382.

[2] A yeast-vegetable extract with ferric citrate.

[2] Hank's solution contains 40 gm. of sodium hydroxide and 2 gm. of potassium alum in 1 liter of water.

agar; and with 43 specimens, *M. tuberculosis* colonies were observed first on the Petragnani medium. Two specimens yielded colonies of *M. tuberculosis* on the charcoal agar while the corresponding cultures on Petragnani medium were overgrown by contaminating organisms. In 116 instances the Petragnani cultures were positive for *M. tuberculosis* and the charcoal agar slants showed no growth. The converse—positive charcoal agar and no growth on Petragnani—occurred with only 7 specimens.

Concerning contamination, 407 of the 7,134 tubes of the Petragnani medium and 102 of the 7,134 tubes of charcoal agar were contaminated. The percentages were 5.7 and 1.4, respectively. If specimens rather than numbers of tubes of media are considered and the result "contaminated" is recorded only when both tubes are affected, then the contamination rates would be 3.5 per cent for the Petragnani medium (124 specimens) and 0.7 per cent for the charcoal agar (25 specimens).

* * *

Of the 206 cultures of *M. tuberculosis* isolated from 3,567 specimens, 116 (56 per cent) would have been missed if only the charcoal agar medium had been used. Conversely, only 9 of the specimens positive for *M. tuberculosis* on charcoal agar were negative or overgrown on Petragnani medium. The charcoal agar was so markedly inferior to Petragnani medium for the isolation of *M. tuberculosis* that the comparison of the contamination rate had little significance.

The favorable results with charcoal agar in the pilot study in which 30 specimens with positive slides were used emphasize the danger in drawing conclusions from an inadequate sampling. It is probable that the amount of inoculum in terms of numbers of organisms was sufficiently large in the pilot study to mask any differences among the several media in growth-promoting properties.

Although every care was taken to duplicate the medium described by Whalen and Mallmann, the possibility is recognized that there could have been some slight but important differences in the media used in the studies in the two laboratories.

The advantages of the addition of charcoal to media designed for the cultivation of tubercle bacilli have been described by a number of workers, notably Hirsch[4] in 1957. The promising results with pure cultures of *M. tuberculosis* should encourage further attempts to incorporate charcoal in a medium for the isolation of the organism in routine diagnostic work.

[4] Hirsch, J. G.: Amer Rev Tuberc, 1954, *70*, 955.

TABLE 1

Results of Cultures for *M. Tuberculosis* on Charcoal Agar and Petragnani Medium

Number of Specimens	*M. Tuberculosis*	
	Charcoal Agar	Petragnani Medium
81	+	+
7	+	−
2	+	Contaminated
116	−	+
3,235	−	−
21	Contaminated	Contaminated
101	—	Contaminated
4	Contaminated	−

\+ = *M. tuberculosis* found.
− = *M. tuberculosis* not found.

TABLE 2

Comparison of Results on Charcoal Agar and Petragnani Medium: 206 Specimens with Positive Results on One or Both Media

M. Tuberculosis Found	Number of Specimens
Both media; same time interval	36
Both media; earlier on charcoal agar	2
Both media; earlier on Petragnani	43
Charcoal agar only; Petragnani contaminated	2
Petragnani only; no growth on charcoal agar	116
Charcoal agar only; no growth on Petragnani	7

In summary: The charcoal agar medium of Whalen and Mallmann was used in comparison with Petragnani medium for cultures for *M. tuberculosis*. Of the 3,567 specimens examined, 90 yielded growth of *M. tuberculosis* on charcoal agar, in comparison with 197 on Petragnani medium. The charcoal agar was found to be markedly inferior to Petragnani medium for the isolation of *M. tuberculosis*.

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NOMOGRAPH FOR CALCULATION OF RESPIRATORY VOLUME RELATIONSHIPS

The calculation of the percentages of the timed vital capacity, the predicted vital capacity, the total lung capacity, and the predicted residual volume is a tedious daily manual or machine operation in most cardiopulmonary laboratories. The calculation must be performed regardless of the type of apparatus used. Testing with the Gaensler-Collins Timed Vitalometer, the 9-liter or 13.5-liter Collins Respirometer, or the McKesson Vitalor all involve the same calculation.

To facilitate the computation, a nomograph has been prepared (figure 1). A straight line drawn from a point on the fractional volume axis and through the total volume axis will intersect the percentage of the timed vital capacity per unit time, the percentage of total lung capacity, or the relationship between any two volumes to be expressed as a percentage. A straight line drawn from a point on the observed volume axis and through the predicted volume axis will intersect the percentage of predicted vital capacity, the percentage of predicted residual volume, or the percentage relationship between any observed volume and the predicted volume.

In summary: A nomograph has been prepared which will provide a convenient method for the rapid solution of respiratory volume relationships and the percentage relationship between any observed volume and the predicted volume.

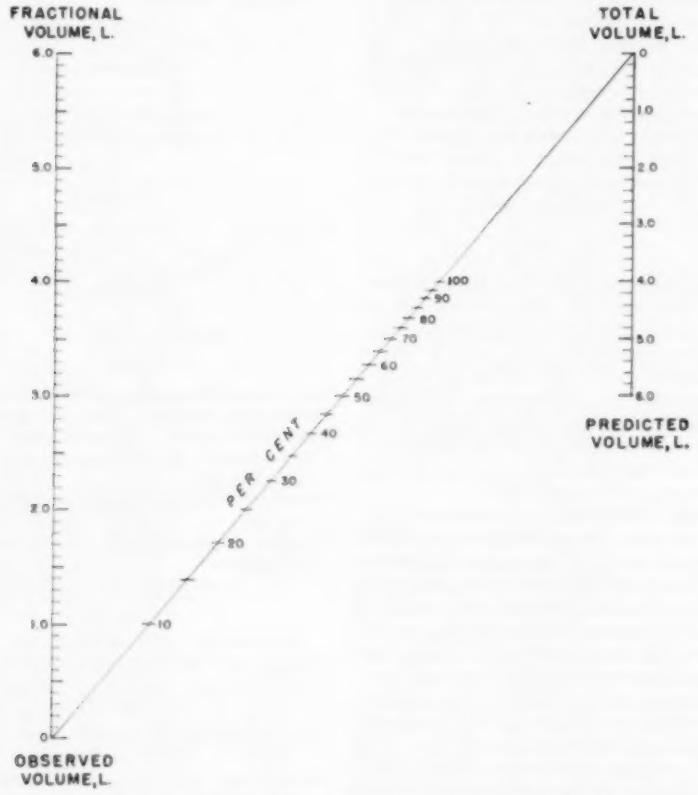

FIG. 1. Nomograph for calculation of respiratory volume relationships.

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A PROPOSED REVISION OF THE GEL DOUBLE DIFFUSION TEST FOR DETECTION OF MYCOBACTERIAL ANTIBODY

Previous reports of gel double diffusion precipitation analyses of serum from tuberculous persons have been concerned with evaluation of test sensitivity and specificity.[1, 2, 3] Each report has contained small percentages of negative test results obtained with known tuberculous serum ("false negatives"), and positive test results with supposedly nontuberculous serum ("false positives").

One of these earlier studies[2] used serum specimens from patients representing a wide range of pathologic conditions. The results indicated that no one disease could be correlated consistently with the false positive tests. Therefore, some other explanation than the fortuitous occurrence of nonspecific antibody in nontuberculous serum was needed to account for false positive results.

It is possible that some of these false positive results can be accounted for on the basis of a mistaken diagnosis of the disease in the serum donor. Occasionally, occult cases of tuberculosis in apparently healthy individuals are eventually recognized and, sometimes, supposedly nontuberculous infections eventually are proved to be tuberculosis.

Another explanation for these false positive results could be made on the basis of a gel double diffusion artifact. Some recent observations have revealed that these artifacts may be inorganic precipitates and that their development is due to poorly controlled pH, since buffering of antigen, agar, and the serum prevents their development. The most recent method for buffering the gel double diffusion test will be given later in the text.

In the gel double diffusion test as previously described,[2] it was observed that some serum specimens positive for tubercle bacilli reacted more rapidly and formed a greater number of bands than did others. Furthermore, if serial dilutions of these specimens were tested identically against a uniform antigen-agar mixture, considerable variation in the appearance of the positive reaction was observed. Some of the bands became more apparent, while others became faint and eventually disappeared as the serum dilution in the gel double diffusion tubes increased. In some cases, as the serum was diluted new bands appeared. If this phenomenon had been simply one of uniform decrease in the degree of positive reaction, it could be attributed to a uniform decrease in the antibody concentration of the test serum dilutions. However, the phenomenon was not uniform, and this indicated that the optimal reaction with each serum required a definite ratio of antigen to antibody in the entire gel double diffusion tube.

Results from tests of 185 known tuberculous serum specimens[4] using four different concentrations of antigen for each serum specimen proved this hypothesis to be true. The four antigen concentrations were, by volume, 75, 50, 25, and 5 per cent antigen in agar. The detailed results of 10 of the 185 specimens given in table 1 illustrate the patterns of results obtained in multiple antigen concentration tests.

In table 1 the serum test results with each of the four antigen concentrations are given as positive or negative, and the total number of positive tests for each is indicated at the bottom of the columns. Five of 10 were positive with the two higher antigen concentrations. With the 25 per cent antigen 6 of 10 were positive, and with the 5 per cent antigen concentration 7 of 10 were positive. If a serum specimen is considered positive when a specific band of precipitate appears in any one or more of the four tubes, then 9 of the 10 specimens were positive to the gel double diffusion test. In the full analysis of all 185 serum specimens it was found that 111 (60 per cent) of the specimens were positive with the 75 per cent antigen concentration, 135 (72 per cent) were positive with the 50 per cent antigen, 132 (71 per cent) were positive with the 25 per cent antigen, 120 (64 per cent) were positive with the 5 per cent antigen, and a total of 170 (92 per cent) were positive at one or more of the four antigen concentration levels. Therefore, a critical ratio of antigen to antiserum concentrations had to be obtained before a visible precipitate would develop. The use of four widely spaced antigen concentrations per serum test enhances the possibility of approaching this equivalence ratio and, therefore, of obtaining positive results with tuberculous serum.

Recommended revised gel double diffusion test procedure: In carrying out the recommended revised test procedure, the following steps are taken:

(*1*) The inner surfaces of 10-cm. × 3-mm. (internal diameter) soft glass tubes are coated

[1] PARLETT, R. C., *et al.*: Amer Rev Tuberc, 1958, *77*, 462.

[2] PARLETT, R. C., AND YOUMANS, G. P.: Amer Rev Resp Dis, 1959, *80*, 153.

[3] LESTER, W., AND COULTON, R.: Trans 18th Conf Chemother Tuberc, VA—Armed Forces, 1959, p. 232.

[4] Kindly supplied by the Suburban Cook County Tuberculosis Hospital, Hinsdale, Illinois.

TABLE 1

MULTIPLE ANTIGEN CONCENTRATION GEL DOUBLE DIFFUSION TEST RESULTS OF 10 TUBERCULOUS SERUM SPECIMENS AFTER 48 HOURS' INCUBATION

Specimen Number	Antigen Concentration in Agar			
	75 Per Cent	50 Per Cent	25 Per Cent	5 Per Cent
1	0	0	+	+
2	0	0	0	+
3	0	+	+	+
4	0	0	+	+
5	+	+	0	0
6	+	0	0	0
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	0	0	0	0
Total positive	5	5	6	7

0 = Negative.
+ = Positive.

with a dried film of agar, as described previously.[2]

(2) A stock agar of 2 per cent in 0.01-M phosphate buffer, pH 7.2, is prepared using ion-agar No. 2 (Oxoid®).[5]

(3) Stock agar is mixed with appropriate dilutions of antigen (made with the 0.01-M phosphate buffer, pH 7.2) to give final antigen-in-agar concentrations of 75, 50, 25, and 5 per cent.

(4) The antigen-in-agar mixtures are used to fill 4.5 cm. of the tubes.

(5) A second 0.5-cm. layer is prepared by diluting stock agar 50 per cent with the 0.01-M phosphate buffer. This is placed on the surface of the gelled antigen-agar column.

(6) Each serum specimen is buffered by adding 0.5 ml. of the 0.1-M phosphate buffer of pH 7.2 to each 5-ml. volume of serum. Each of the four different antigen concentration tubes is then filled with this buffered serum.

(7) The tubes are incubated in a closed container at 37°C., and are observed at twenty-four, forty-eight, and seventy-two hours for the presence or absence of specific precipitates.

Recording and analyzing gel double diffusion test results: Following 37°C. incubation, positive gel diffusion tests develop sharp, distinct bands of precipitate in the center agar layer. The intensity of the bands ranges from very faint to very dense lines. If a given band appears in more than one of the four antigen concentration tubes, the location of that band within the center columns may vary. This difference in location is due to altered antigen diffusion rates as influenced by the antigen concentrations in the four tubes.

As the incubation time increases to twenty-four, forty-eight, and seventy-two hours, the individual bands of precipitate change in appearance. Some become more dense and retain their sharp delineation, others intensify but develop a ragged appearance, still others become slightly more diffuse, and some even disappear. These changes can be attributed to both the characteristics of the individual antigens and to altered concentration levels within the center column. Apparently, deterioration and/or disappearance of some bands is due to an accumulation of either excess antigen or antibody within this reaction area.

If excess antigen accumulates in the center column before sufficient concentrations of antibody are reached, initial precipitation may be inhibited (*serum specimens 1–4* in table 1). Conversely, high concentrations of antibody may build up in the center column before a sufficient quantity of antigen appears, and they also may inhibit the initial precipitation (*serum specimens 5* and *6* in table 1).

If the gel double diffusion test results are recorded in the manner illustrated in table 1, the serum specimens fall into one of four categories:

(1) Serum specimens which are negative with all antigen concentrations

(2) Serum specimens which are positive with all antigen concentrations

(3) Serum specimens which are positive with higher and negative with lower antigen concentrations

(4) Serum specimens which are negative with higher and positive with lower antigen concentrations

Serum specimens in *category 1* can be regarded as antibody negative. Serum specimens in *category 2* can be regarded as possessing moderate antibody titers since no inhibition occurs. Serum specimens in *category 3* are apparently of high antibody concentration since the high antigen levels do not inhibit precipitation, whereas precipitation is inhibited in the lower antigen concentration tubes. The specimens in *category 4* can be considered to have very low antibody titer as the higher antigen concentrations do appear to inhibit visible precipitation.

Frequently a flocculate is observed to develop in the overlying antiserum which increases in amount and settles onto the center layer surface as incubation time increases. This flocculate seems to develop at the time when antigen diffuses through the center agar layer and enters the antiserum. In a series of gel double diffusion tests in

[5] Manufactured by Consolidated Laboratories, Inc., Box 423, Chicago Heights, Illinois.

which the antigen concentration was held constant and twofold antibody dilutions were tested, it was noticed that the end point of this flocculation phenomenon lagged behind the end point of the gel double diffusion precipitate reactions by several twofold dilutions. This result is consistent with the theory that gel double diffusion precipitation is more sensitive than the flocculation reaction.

In summary: A revised method is given for performing the gel double diffusion precipitation test for mycobacterial antibody. The revision includes a new, more complete buffering of the gel diffusion reagents and a substitution of ion-agar No. 2 for the agar base, and recommends the use of at least four antigen concentrations for testing each serum specimen. Data are included to illustrate the value of using multiple antigen concentration. Some description and discussion are given of the appearance of positive bands of precipitate, and the manner in which the factors of time and change in relative antigen and antiserum concentrations may alter their appearance.

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Abstracts of Articles in Volume 23, No. 4–5, 1960, by Agnes Fahy[1]

ANTIMICROBIAL THERAPY OF PULMONARY TUBERCULOSIS

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Various changes have taken place in the management of pulmonary tuberculosis since the first successful demonstration of the antimicrobial therapy of the disease approximately fifteen years ago. It is appropriate that we take stock of these changes at this time.

Among the questions that have arisen are those of hospital vs. home care, which also brings up the question of bed rest vs. ambulation; the choice of antimicrobial agents for previously untreated patients; the management of patients who have not been treated successfully; the "open negative" syndrome; the duration and dosage of chemotherapy; the use of steroids with antimicrobials; the locus of action of two-drug therapy; implications of drug resistance; and collapse and excisional therapy. These questions will be considered in order.

Hospital versus Home Treatment

Since antimicrobial agents in use are not eradicative, chemotherapy must be prolonged, for perhaps two or three years. Part of this period will inevitably be at home, with antimicrobials self-administered and only periodic supervision by physicians or other personnel.

Tuberculous patients are hospitalized to provide them with supervised bed rest and to isolate the infectious patient. As for bed rest, two recent clinical studies show that, with theoretically adequate chemotherapy, it is impossible to detect any influence of physical activity on the generally favorable healing process of the tuberculous lesion. The studies were conducted at the Rockefeller Institute for Medical Research in New York (published in 1957) and at the Tuberculosis Chemotherapy Centre, Madras, India (published in 1959).

As for isolation, there has never been a time or place when all patients were hospitalized until noninfectious. Certainly today, when bed rest is not considered necessary, it would lead to a difficult problem in management. Therefore, the decision of whether or not to keep the patient in the hospital must be made primarily on the basis of the nature of the lesion and the conditions surrounding the patient. If possible, patients with highly infectious lesions, such as laryngeal disease, should be kept in the hospital, while patients with chronic cavitary lesions need not be kept in the hospital indefinitely unless the household associates would be at great risk.

With adequate chemotherapy, reversal of infectiousness occurs usually within the first three to six months. If isoniazid is used in treatment, the risk of household associates would not only be limited to that period but would be further reduced by the fact that a portion of the tubercle bacilli are probably biologically altered toward lessened pathogenicity. In some situations, however, BCG vaccination for household associates would be indicated.

Choice of Chemotherapy for Previously Untreated Patients

The four regimens available for initial chemotherapy are: isoniazid and streptomycin daily, isoniazid and para-aminosalicylic acid (PAS) daily, isoniazid alone, or isoniazid with twice- or thrice-weekly streptomycin. Another regimen, pyrazinamide-isoniazid, while effective, cannot be recommended for general use because of the toxicity of pyrazinamide.

Isoniazid and daily streptomycin represent the most effective therapy per unit of time, with isoniazid-PAS almost as effective. There is probably little difference between isoniazid alone and isoniazid and streptomycin twice a week. In all of these regimens, isoniazid provides the major antituberculosis action.

Of the three drugs in widespread use, PAS has the greatest toxicity. In addition to gastrointestinal intolerance, there are other toxic manifestations, notably the hypersensitivity phenomenon with the syndrome of infectious mononucleosis.

While isoniazid with streptomycin less than daily appears slightly superior to isoniazid alone, the difference is so slight that, in view of the difficulties of the administration of the streptomycin to ambulatory patients, it is probably as well to use isoniazid alone.

For all practical purposes, the choice of therapy for the patient outside the hospital is between isoniazid-PAS and isoniazid alone, and the margin

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of difference between these two regimens becomes the most important question in the chemotherapy of tuberculosis today.

Reasonable indications for the use of isoniazid alone are found in the following situations: (*1*) as prophylaxis in the tuberculin reactor without other evidence of disease; (*2*) as initial treatment in noncavity pulmonary tuberculosis; (*3*) as follow-up treatment after reversal of infectiousness and cavity closure; (*4*) as initial treatment in cavitary pulmonary tuberculosis in certain circumstances, such as in places with high prevalence when the additional expense and problems created by the use of PAS would mean restriction of the program, and in patients in whom the prospect of cavitary closure is good but who cannot take PAS and for whom daily streptomycin is not feasible.

When the patient with moderately or far advanced disease is treated on one of the four regimens outlined, the disease takes one of the following courses:

1. There is considerable regression of pulmonary infiltration, closure of cavities, and attainment of a noninfectious state. This occurs in the majority of cases.

2. There is regression, failure to close cavity, but attainment of noninfectiousness. This is the "open negative" syndrome and occurs in 15 to 20 per cent of the cases.

3. There is greater or lesser regression of infiltration, but no impressive reduction in cavity size, and the discharge of tubercle bacilli continues. These are the chemotherapeutic failures and probably represent about 15 per cent of the cases.

Management of Previously Unsuccessfully Treated Patients

Factors in addition to *in vitro* drug resistance are involved in the failure of cavities to close. Since bacilli proliferate in the caseous material lining the cavity wall, the cavity itself may become a "cause" of drug resistance. With persistence of cavity, the factors that led to treatment failure are still operative and may continue to impede the effectiveness of other chemotherapy.

Treatment should ideally be in the hospital where collapse or excisional therapy can be employed to obliterate cavities. While a change in chemotherapy is indicated, it may be wise to continue to use isoniazid despite *in vitro* evidence of resistance to this drug. The second drug can be chosen from four now available: cycloserine, pyrazinamide, α-ethylthioamide, and viomycin. Of these, viomycin is probably the least effective. The others are administered by mouth but should not be used on a widespread basis outside the hospital because of toxicity. Use should be limited to three or four months.

Used with isoniazid, cycloserine in two daily doses of 250 mg. each appears to be highly effective; any increase in a single dose raises the danger of neurotoxicity. A new derivative in a series studied for the past few years, α-ethylthioamide appears to be as effective as PAS, streptomycin, or cycloserine as a companion to isoniazid, but nausea and vomiting are a problem. There is some evidence, too, that the cumulative effect of thioamide with isoniazid leads to the production of peripheral neuritis.

Pyrazinamide has limited effectiveness alone, but is highly effective with isoniazid or streptomycin. However, about 3 per cent of patients receiving the drug develop hepatitis with jaundice. It should, therefore, be employed only when the risk from the disease clearly outweighs the toxic potential of the drug. A U. S. Public Health Service study has indicated that pyrazinamide with daily streptomycin for 12 weeks, followed by isoniazid-PAS, is an extraordinarily effective regimen. However, the danger of hepatitis places a limitation on its usefulness.

Viomycin and the neomycin series, which includes kanamycin, have the same pattern of toxicity as streptomycin, but are more toxic and less effective.

Therapy with the secondary drugs, particularly without isoniazid, should never be instituted solely on the basis of drug-resistance tests. There must be evidence of persistent cavitation and an unfavorable trend of the disease. Otherwise, the patient may be exposed to greater risk from drug toxicity than from the disease itself.

Management of Patients with Open-negative Syndrome

The "open negative," in which cavitation persists even though healing has occurred, was seen but rarely in the days before chemotherapy. Usually, surgery is unnecessary. The decision whether or not to excise should be based on the circumstances surrounding the individual patient. A young physician or dentist with an "open-healed" cavity should probably have it removed, while an older person who would not be a public health threat might not need surgery but should continue to take antimicrobials.

Duration and Dosage of Chemotherapy

Chemotherapy is usually administered for eighteen to twenty-four months. The principal action of the drug may be exerted in the early weeks; thereafter its action is protective. However, tu-

bercle bacilli are not eradicated as a direct consequence of chemotherapy, certainly not within the first half year of therapy; therefore, it is wise to continue drugs well past the time when most of the healing takes place.

As for dosage: With streptomycin a dose of 20 mg. per kg. per day (1 gm. for the average-sized adult) appears to be within the safe limits to avoid vestibular toxicity. The dosage of PAS depends to some extent on the dosage of isoniazid, when it is used with that drug. It is probable that with 300 mg. or more of isoniazid daily, the proper dosage of PAS would be between 6 and 10 gm. a day. The ideal dosage for isoniazid is the maximal tolerated dose. This is usually between 5 and 8 mg. per kg. per day, or a daily dose between 300 and 450 mg. for an adult patient. The question of high dosage for slow inactivators of isoniazid has been studied, but the evidence indicates that the number of high acetylators, that is, those who inactivate the drug rapidly, is small and that, even in these cases, the drug is present in its active form for several hours before acetylation becomes excessive, by which time isoniazid will have exerted its maximal activity. The point about isoniazid is that it is advisable to give as large a dose as possible within safe limits. Thus, the relatively unusual individual with high acetylation will be protected from excessive neutralization of the drug while the majority will be protected against excessive toxicity. Pyridoxine is not indicated with isoniazid. Reduction of neurotoxicity when pyridoxine is administered with high-dosage isoniazid appears to take place at the expense of the effectiveness of isoniazid.

Steroid Hormones with Antimicrobial Therapy

Tubercle bacilli multiply in tissue altered by steroid hormones to a greater extent than they do in unaltered tissue. The effectiveness of antimicrobial drugs is neither enhanced nor lessened by the administration of the hormones. Since the side effects of steroid hormone administration are well known, they should be avoided if possible except in extreme cases in which the immediate action may militate in favor of the patient on the verge of death.

Locus of Action of Two-Drug Therapy

Two-drug therapy appears to be superior because both drugs act on microbial cells susceptible to both drugs. This concept does not imply that emergence to predominance of drug-resistant genotypes would necessarily remain unaffected. But, it is considered unlikely that the conventional explanation of how this is accomplished (by action of one drug on cells genetically resistant to the other) is correct. Whether the retardation of significant drug resistance occurs by the mechanism postulated or by the genetic mechanism, the fact is that some strains of tubercle bacilli resistant to one or both of the drugs employed eventually emerge. Thus, multiple-drug therapy, although beneficial, is not a guarantee against the emergence of drug-resistant infections. It is not without interest that a majority of the drug-resistant strains of tubercle bacilli isolated in presumably untreated infections are resistant to more than one drug.

Implications of Drug Resistance

Two questions are important in regard to drug resistance: reduction in therapeutic effectiveness and the epidemiologic implications of the spread of drug-resistant infections in a community.

Thus far, there is little indication that strains resistant to isoniazid, the most widely used drug, constitute a public health threat of any significance. Nevertheless, the threat exists, and careful epidemiologic "monitoring" should be maintained.

One of the questions that must be answered in regard to resistance to any drug is whether the presence of the resistant bacilli as shown by *in vitro* tests means that the benefits of that drug are always seriously compromised in the patient. Certainly it does with streptomycin. The situation with regard to isoniazid is not so clear.

It has long been known that tubercle bacilli from isoniazid-treated patients are changed in many particulars and that isoniazid-resistant strains have lowered pathogenicity for guinea pigs. It has also been shown that loss of pathogenicity can be correlated with another biologic property of the bacilli, that is, their ability to form the enzyme, catalase. However, not all catalase-negative strains are completely without pathogenicity. Thus, it may be said that loss of effectiveness due to isoniazid-resistant bacilli does not occur frequently with isoniazid but does occur often enough to be considered in the management of the individual patient who persistently discharges tubercle bacilli after the first three or four months of isoniazid therapy.

Because of the complicated nature of the situation with isoniazid, the effectiveness of the therapy must be determined by means other than susceptibility tests, including observation of the course of infection with isoniazid therapy. There is also the problem of the significance of isoniazid-resistant bacilli for associates of the patient. Some people will be infected by such organisms, but such infections do not seem to have appeared in appreciable numbers yet.

Since the introduction of isoniazid, far more people have probably died because they did not

receive the drug than have been seriously affected, one way or another, by isoniazid-resistant bacilli.

Collapse and Excisional Therapy

Since the conditions of an open cavity are ideal for the proliferation of tubercle bacilli and, hence, for the emergence to predominance of drug-resistant strains, the elimination of cavities would be an important measure to avoid drug resistance. Surgical excision is not always advisable; therefore, it may be wise to turn again to pneumothorax and pneumoperitoneum. With the availability of drugs, many of the problems that formerly arose with pneumothorax would not be encountered. The procedure lends itself well to ambulatory treatment and might be a highly satisfactory additional measure to the chemotherapy of tuberculosis.

Prevalence of Tuberculosis among Close Family Contacts of Tuberculous Patients in South India, and Influence of Segregation of the Patient on the Early Attack Rate

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A controlled study to compare the results of the treatment of pulmonary tuberculosis in the home and in the sanatorium, with patients on the same drug regimens, was conducted by the Tuberculosis Chemotherapy Centre, Madras, India. A report on the study, published in 1959, concluded that "the results of domiciliary chemotherapy, as carried out in this study, approach sufficiently closely the results of sanatorium treatment to suggest that it is appropriate to treat the majority of patients at home."

The present study was undertaken to determine the prevalence of tuberculosis among the close family contacts of both the domiciliary and sanatorium patients and the incidence of clinical tuberculosis and tuberculous infection in the contacts during the first year in which the patient, or index case, was under treatment.

Plan of Study

In the original study, the patients were from 191 families living under crowded urban conditions in Madras City, in South India. They had 693 close contacts, divided almost evenly (347 and 346, respectively) between male and female. Nearly half were less than fifteen years of age, 19 per cent were less than five years of age, and only 6.1 per cent were fifty-five or older.

At the time the index case was diagnosed the contacts were examined roentgenographically and were given Mantoux tuberculin tests with 5 tuberculin units (TU) of PPD. If there was no induration or if the induration was less than 5 mm., another test was made with 100 TU. The PPD used was batch RT 22, supplied as powder by the Statens Seruminstitut, Copenhagen, Denmark. The diluent was phosphate buffer and Chinosol. It should be noted that this batch of tuberculin is somewhat weaker than the International Standard PPD.

In following up the contacts, roentgenograms of the chest were obtained at three, six, and nine months and at one year. The tuberculin test was also repeated on all persons whose initial test with 5 TU was less than 20 mm. in induration. This was done in order to obtain as complete a picture as possible of changes in tuberculin sensitivity.

None of the contacts had had BCG.

Prevalence

There were 46 cases of active tuberculosis among the contacts at the time the index case was diagnosed. This was 6.8 per cent of the 672 family contacts with initial chest films. Twelve of the active cases were among 131 children less than five years of age and only one was among 145 contacts between the ages of ten and nineteen. There were seven cases among 47 contacts between twenty and twenty-four years of age and nine among 48 between forty-five and fifty-four.

Attack Rate

For various reasons, such as the presence of two or more family members with infectious sputum, an abnormality on the initial chest film, or the lack of an initial chest film, some of the contacts had to be excluded in determining the attack rate of tuberculosis. There remained 532 close family contacts from 148 families, which broke down to 257 contacts of 75 patients treated at home and 275 contacts of 73 patients treated in the sanatorium.

In the home series, 201 of the 257 contacts continued to live, cook, and eat with the person who was the index case.

Of the sanatorium contacts, 261 (94.9 per cent) had, for all practical purposes, no contact with the index case. The remaining 14 (5.1 per cent) had renewed contact when the index case was prematurely discharged from the sanatorium.

In the initial tuberculin tests with 5 TU, 35.0

[a] Tuberculosis Chemotherapy Centre, Madras, India. The Centre is under the joint auspices of the Indian Council of Medical Research, the Madras State Government, the World Health Organization, and the Medical Research Council of Great Britain.

per cent of the home contacts and 32.7 per cent of the sanatorium contacts had indurations of less than 5 mm. and were considered negative. Indurations of 5 to 7 mm., considered a weak positive, occurred in 18.7 per cent and in 14.7 per cent of the home and sanatorium contacts, respectively, and indurations of 8 to 9 mm., considered definitely positive, were found in 9.8 per cent and in 9.0 per cent of the home and sanatorium contacts.

A total of 26 of the contacts developed active tuberculosis during the year. The 26 cases consisted of 7 in 86 home contacts and 5 in 87 sanatorium contacts who were originally tuberculin negative; one in 46 home contacts and 2 in 39 sanatorium contacts who had been weakly positive, and one in 114 home contacts and 10 in 140 sanatorium contacts who had originally had indurations of 8 mm. or more.

While the incidence of tuberculosis was similar in the two groups among those who were initially tuberculin negative and among those with positive reactions, it was slightly higher among the sanatorium contacts than among the home contacts. The difference is presumably due to chance, but even so it is surprising since the index cases of the home contacts as a group had more extensive disease on pretreatment roentgenography than did the sanatorium index cases.

Conversion of tuberculin tests from negative to positive (defined as an increase of at least 10 mm. in a 5 TU test that was originally negative) occurred at similar rates in both groups of contacts, with possibly a slightly higher rate among the home contacts. The fact that some of the sanatorium contacts converted in the latter six months suggests that there were sources of infection outside the family. This was undoubtedly true also of the home contacts.

Discussion

The prevalence rate—6.8 per cent of the contacts at the time the index case was diagnosed—is higher than that in the general Indian population in large cities as revealed in the national sample survey between 1955 and 1958, which showed that the prevalence of "active and probably active tuberculosis" in those five years old or more ranged from 1.5 per cent to 2.1 per cent in six cities. The prevalence of active tuberculosis in these age groups in the contacts study was 6.3 per cent.

Among the home contacts, tuberculosis developed in the last six months in 4 people who were initially tuberculin-negative. No tuberculosis developed among the initially negative sanatorium contacts in the second six months.

If these findings are representative of known contacts in urban India, it may be advisable to give antituberculosis chemoprophylaxis to all young household contacts of newly diagnosed sputum-positive patients living in overcrowded urban conditions, even if the contacts have normal chest roentgenograms, whether or not they react to the tuberculin test and whether the index case is segregated in the sanatorium or treated at home. It is suggested that a minimal period of six months would be desirable, and that isoniazid might be used alone if a suitable companion drug were not available.

The precise role of BCG vaccination for the contacts is not clear. Since it has been shown that BCG confers substantial protection for tuberculin-negative subjects for a number of years, BCG would be a desirable addition to the limited duration and immediate protection of chemoprophylaxis. However, protection might still develop too late to benefit tuberculin-negative contacts who have either already been infected or are on the verge of infection at the time of vaccination. Moreover, the development of protection might be hindered if prophylactic isoniazid were administered to those just vaccinated with isoniazid-susceptible BCG. This possible disadvantage might be overcome if an isoniazid-resistant BCG vaccine could be used, as has been suggested by Georges Canetti, but such vaccines are still in the experimental stage. Thus, it is uncertain whether BCG might be effectively given at the beginning of a course of chemoprophylaxis or should await its end.

Progress in the Second Year of Patients with Quiescent Pulmonary Tuberculosis after a Year of Chemotherapy at Home or in Sanatorium and Influence of Further Chemotherapy on the Relapse Rate

S. Velu,[4] R. H. Andrews,[4] S. Devadatta,[4] Wallace Fox,[4] S. Radhakrishna,[4] C. V. Ramakrishnan,[4] J. B. Selkon,[4] P. R. Somasundaram,[4] and T. V. Subbaiah[4]

As an outgrowth of the study sponsored by the Tuberculosis Chemotherapy Centre, Madras, India, to compare results of one year of drug treatment of patients with pulmonary tuberculosis in the sanatorium and at home, a group of the patients were followed up with an additional year of treatment at home.

In the original study, isoniazid and para-aminosalicylic acid (PAS) had been given both sanatorium and home patients. It was found that there was little difference in the response to treatment between domiciliary and sanatorium patients.

The purpose of the study here reported was to

[4] Tuberculosis Chemotherapy Centre, Madras, India.

determine (*1*) whether bacteriologic relapse and roentgenographic spread of disease occurred more frequently in patients who had attained quiescence of disease at home than in those who had been treated in the sanatorium; and (*2*) whether a second year of chemotherapy with isoniazid alone, after a year of combined therapy, would bring results superior to those attained with one year of treatment.

It was hoped, too, to determine the effect of the second year of treatment of patients with residual cavitation, that is, the "open negatives," and the progress of patients whose bacteriologic status had been doubtful at the end of the first year because of an isolated positive culture late in the course of treatment.

Selected for the study were 126 patients, 57 of whom had been treated at home and 69 of whom had been treated in a sanatorium. All had attained bacteriologic quiescence. The patients were randomly allocated for isoniazid (65 patients) or for placebo consisting of calcium gluconate (61 patients). Of the total, 42 were "open negatives" and 84 showed no evidence of residual cavitation.

The isoniazid dosage was 200 mg. a day, taken as one pill, if the patient weighed 100 pounds or more. A smaller dose was given patients who weighed less. Dosage was increased with weight gain but was not decreased with weight loss.

The patients picked up their drugs monthly at the Centre, where they had routine examinations. Their homes were usually visited twice a month, once to deliver a bottle for a sputum specimen, once without notice to check on how well drugs were being taken. Checks revealed that patients who had originally been on home treatment were slightly less regular in taking their pills than were the former sanatorium patients. There was no difference between patients on placebo and isoniazid in this respect.

In addition to the 126 patients, there were 10 patients whose bacteriologic status was in doubt because of an isolated positive culture near the end of the first year of treatment and whose progress was analyzed. Seven of these were from the home series and 3 from the sanatorium. Treatment was changed for one of the sanatorium series during the second year. The other 9 were bacteriologically quiescent at the end of the second year.

Comparative results: Chemotherapy was changed during the year for one of the home and for 4 of the sanatorium patients because of roentgenographic evidence of deterioration and bacteriologic relapse. One home patient, with cavitation and on isoniazid, died. The cause of death was not clearly established. Three of the 5 whose treatment was changed had been on placebo, two on isoniazid.

No changes in roentgenographic appearance were found in the second year in 81 and 80 per cent of the home and sanatorium patients, respectively. Some degree of roentgenographic improvement was noted in 9 per cent of the home and in 12 per cent of the sanatorium patients. Slight roentgenographic deterioration occurred in 7 per cent of the home and in 3 per cent of the sanatorium patients.

For the two-year period, 84 per cent of the home and 87 per cent of the sanatorium patients had at least moderate improvement demonstrated roentgenographically.

The radiologic picture viewed in relation to placebo and isoniazid patients shows that 5 in the placebo group and 8 on isoniazid improved slightly while 7 on placebo and 4 on isoniazid had roentgenographic deterioration. No change in roentgenographic appearance was found in 80 per cent in each group.

Of the 36 home patients and 48 sanatorium patients who appeared to have no cavitation at the end of the first year, one in each group had slight cavitation at the end of the second year. Both of these patients were on placebo the second year.

There were 19 home and 19 sanatorium patients with residual cavitation at the beginning of the second year. Two of the home and 4 of the sanatorium group had no cavitation at the end of the second year while 12 of the home and 4 of the sanatorium patients had less cavitation. Of the 38 open negatives, 18 were on calcium gluconate and 20 on isoniazid. Cavitation disappeared in 2 of the former and in 4 on isoniazid, while cavitation was reduced in 7 and 9 on placebo and isoniazid, respectively. There was no change in 5 on placebo and 4 on isoniazid, and cavitation increased in 4 on placebo and in 3 on isoniazid.

Negative cultures only were yielded during the second year by 52 (91 per cent) of the 57 home patients and by 61 (88 per cent) of the 69 sanatorium patients. Three (5 per cent) of the home and 2 (3 per cent) of the sanatorium patients each had a single positive culture. Isolated positive cultures were uncommon and multiple positive cultures occurred in 8 patients, 5 of whom showed roentgenographic deterioration.

When bacteriologic status is considered in relation to placebo and isoniazid, it is found that 54 (89 per cent) of the calcium gluconate series and 59 (91 per cent) of the drug series had all had negative cultures during the second year, while 2 on placebo (3 per cent) and 3, including the one who died (5 per cent) on isoniazid had had one positive culture. The remainder had had more than one positive culture.

Discussion

No evidence is found in this study that continuing chemotherapy with isoniazid alone in quiescent patients for a second year brings better results than those obtained at the end of a year of treatment with combined drugs.

It is possible that two years of combined chemotherapy would give better results, but combined chemotherapy was not practicable in the situation discussed, and there is no prospect in the foreseeable future that such therapy will be available for widespread use in the underdeveloped countries for one year, much less two. However, the 7 per cent relapse rate for patients who had had only one year of chemotherapy, that is, those who were on placebo the second year, is relatively low.

While the open negatives did not do as well as those without residual cavitation, there was no evidence that the use of isoniazid alone in the second year was capable of preventing relapse in patients with cavitated lesions. (Other reports on the open negative syndrome will be made later.)

Conclusion

Since relapse rates in the second year for patients treated at home and in the sanatorium were both small, it would appear that a second year of chemotherapy with isoniazid alone does not apparently confer further benefit.

A Concurrent Comparison of Isoniazid plus PAS with Three Regimens of Isoniazid Alone in the Domiciliary Treatment of Pulmonary Tuberculosis in South India

TUBERCULOSIS CHEMOTHERAPY CENTRE, MADRAS

To compare the merits of four drug regimens in the domiciliary treatment of pulmonary tuberculosis, a controlled study was conducted by the Tuberculosis Chemotherapy Centre, Madras, India. This report deals with the findings at the end of the first twelve months.

Three regimens of isoniazid alone were compared with a regimen of isoniazid and para-aminosalicylic acid (PAS). The regimens were as follows:

H: 200 mg. of isoniazid daily, the dosage being divided into eight tablets, four taken in the morning and four in the evening.

HI-1: 400 mg. of isoniazid daily divided into eight tablets, all of which were taken in the morning.

HI-2: 400 mg. of isoniazid divided into eight tablets, four taken in the morning and four in the evening.

PH: 200 mg. of isoniazid plus 10 gm. of PAS (sodium salt) divided into eight cachets taken twice a day.

(The isoniazid given is based on 100 pounds of body weight. The dosage in each case was adjusted to the weight of the individual patient.)

Only patients with pulmonary tuberculosis were admitted to the study, and the assignment of regimens was by random allocation. Other specifications were that the patients had not previously been treated with antituberculous drugs or, at the most, had not been treated for more than two weeks; they had sputum positive for tubercle bacilli; they were at least twelve years old, lived in a specified area of Madras City and were likely to remain there for several years; they agreed to accept home treatment, including drugs, for at least one year, and to permit visits by a home visitor. Furthermore, their families were judged to be cooperative. Patients too ill to be treated at home were not accepted. Excluded also were patients known to have leprosy or other serious diseases and patients known to be pregnant.

There were 341 patients from a poverty-stricken section of a large urban community selected originally. Of these, 22 were excluded from the study because they were known to have had isoniazid-resistant organisms in the pretreatment culture; 4 were excluded for various other reasons such as carcinoma of the bronchus, treatment for more than two weeks, self discharge, and mistake in the chemotherapy. The allocation on treatment regimens was as follows: H—87; HI-1—70; HI-2—68; PH—90.

The patients were treated in their own homes, but the majority attended the Centre weekly to obtain supplies of drugs. The home was visited each week during the first month by a health visitor; less frequently, by a doctor or a public health nurse. One visit a month was unexpected, at which time the stock of drug or drugs was counted and a urine specimen was collected for testing to determine whether the patient was taking his medicine.

All patients had monthly chest films, sputum examination on smear and culture, and various tests, including drug-susceptibility tests. Although streptomycin-susceptibility tests were not made during treatment, streptomycin-susceptibility tests were made on all patients prior to treatment. Eleven of the 341 patients originally considered for the study had streptomycin-resistant strains. It seems probable that they had been infected with such strains. This represents a prevalence of primary streptomycin resistance of 3.3 per cent.

Toxicity: Among the 90 PH patients, three became hypersensitive to PAS in the second month. In one case, it was necessary to change the PAS to streptomycin.

Twenty patients developed peripheral neuritis attributed to isoniazid, the largest percentage being in the HI-1 group (19 per cent) and the smallest in the PH group (1 per cent). In only 7 of the patients was chemotherapy changed. Serum isoniazid assays were performed for the majority of patients and it was found that the patients who developed peripheral neuritis had higher mean serum concentrations of the drug than those who did not develop this complication.

Isoniazid susceptibility: Some resistant cultures were found at one month in all of the series treated on isoniazid alone. By the end of twelve months, 52 of 53 positive cultures among all of the treatment series were resistant, the one exception being a strain in an HI-1 patient.

Rest and activity: Patients were urged to rest as much as possible in the early weeks of treatment. As their condition improved they were allowed to increase their activity, and many were in their normal full-time activity at the end of the year.

Results at twelve months: The progress of the PH series throughout the twelve months was very satisfactory in regard to both roentgenographic improvement and bacteriologic conversion.

The HI-1 series achieved almost the same order of roentgenographic improvement, but sputum "conversion" occurred in 14 per cent fewer patients than in the PH group. The progress of the other two series was less satisfactory.

Roentgenographic changes over the first six months, as assessed by an independent reader, Dr. Raj Narain, showed moderate or greater improvement in 79 per cent of 87 PH patients; 73 per cent of 67 HI-1 patients; 62 per cent of 66 HI-2 patients, and 69 per cent of 86 H patients. For the full twelve months, the majority of the patients showed moderate or greater improvement: 85 per cent of the PH, 78 per cent of the HI-1, 71 per cent of the HI-2, and 62 per cent of the H series. The proportion of patients who showed roentgenographic worsening or who died of tuberculosis during the period was 3 per cent, 8 per cent, 24 per cent, and 28 per cent, respectively.

Cavities disappeared in about a quarter of the small number of patients who originally had extensive cavitation and in almost two thirds of the PH patients with moderate cavitation, and in more than 50 per cent of the HI-1 series and in less than 40 per cent of the others with moderate cavitation.

In all of the series there was a rapid decline in the proportion of patients with cultures positive for tubercle bacilli. By three months 78 per cent of 81 PH patients, 64 per cent of 69 HI-1 patients, 55 per cent of 64 HI-2 patients, and 43 per cent of 82 H patients had negative cultures. At six months the percentages had increased to 88, 71, 58, and 49, respectively. Slight increases occurred thereafter.

A patient's disease was classified as bacteriologically quiescent if all of the cultures were negative for tubercle bacilli for at least the last three monthly examinations. Thus, 86 per cent of the PH patients, 67 per cent of the HI-1, 56 per cent of the HI-2, and 44 per cent of the H patients were classified as bacteriologically quiescent at twelve months, while 5 per cent of the PH, 6 per cent of the HI-1, and 2 per cent of the HI-2 were of doubtful status.

Patients whose disease had relapsed bacteriologically, who had active disease, or who died of tuberculosis were defined as having an unfavorable response. They accounted for 9 per cent of the PH, 27 per cent of the HI-1, 42 per cent of the HI-2, and 56 per cent of the H series.

The differences between the PH and the other three series are statistically highly significant. It may be concluded that the PH series fared well and the progress of the other three series was less satisfactory, particularly that of the HI-2 and H series.

By Title

Peripheral Neuritis Due to Isoniazid. S. Devadatta, P. R. J. Gangadharam, R. H. Andrews, Wallace Fox, C. V. Ramakrishnan, J. B. Selkon, and S. Velu.

A Comparison of the Sensitivity to Para-Aminosalicylic Acid of Tubercle Bacilli from South Indian and British Patients

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As part of the investigation of the chemotherapy of tuberculosis in India undertaken by the Tuberculosis Chemotherapy Centre, Madras, a study was made to compare the sensitivity of South Indian and British strains of tubercle bacilli to para-aminosalicylic acid (PAS) to determine the nature of any differences in PAS sensitivity in the strains.

That there were differences had been suspected because an earlier study had revealed a higher average level of pretreatment PAS resistance among Indian patients than among British patients. Furthermore, PAS resistance did not ap-

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pear to be related as closely to subsequent emergence of isoniazid resistance in Indian patients as in British patients when the two drugs were used in treatment.

In the studies reported, sputum specimens from the South Indian patients, none of whom was believed to have had more than two weeks of previous drug therapy for tuberculosis, were cultured in the laboratory of the Tuberculosis Chemotherapy Centre (hereinafter referred to as TCC). Specimens from previously untreated British patients were cultured at the Postgraduate Medical School of London (PGMS). The same methods were used in both laboratories.

For the sensitivity tests, slopes were set up containing various concentrations of sodium PAS hydrate (0.25, 0.5, 1, 2, 4, 8, 16, and 64 γ per ml.) and were inoculated with 10^6 viable units of organisms. Minimal concentrations of PAS inhibiting growth (MIC) were read at 20-colony, 50-colony, and 100-colony end points.

Three investigations are reported. One was a general comparison of PAS sensitivity of tubercle bacilli from Indian and British patients made at TCC; another was a similar study made at the PGMS; and the third was carried out simultaneously in both laboratories.

Investigation 1: A total of 133 strains from 81 Indians and 52 British patients were examined over a period of five months at the TCC, the strains from British patients having been flown to India.

On the basis of the 20-colony MIC, the Indian strains were on the average more resistant than the British. Values of 4 γ per ml. or more were given by 38 (47 per cent) of the 81 Indian strains and by only one (2 per cent) of the 52 British strains. The differences were less marked when the 100-colony end point was used.

Investigation 2: A total of 87 strains, from 56 Indian and 31 British patients, were tested for PAS sensitivity at the PGMS. Results similar to those noted at the TCC were observed.

The differences between the results and the 20-colony MIC and the 100-colony MIC in this investigation and the first one suggest that some of the Indian strains contained a small proportion of bacilli capable of growth on slopes with the higher PAS concentrations.

Investigation 3: The purpose of the third investigation was twofold: first, to find out whether the difference in the PAS sensitivity of Indian and British strains would still be apparent if the tests were carried out on recently isolated strains which had not been subcultured; and, second, whether the presence of PAS-resistant organisms in Indian strains was a characteristic of all Indian strains or only of strains from particular patients, or whether other factors were involved.

Two sputum specimens obtained within a few days of each other from each of two previously untreated Indian patients were cultured at the TCC and two similarly obtained specimens from British patients were cultured at the PGMS. One slope from each culture was retained at the laboratory which cultured the sputum and another was sent by air to the other laboratory.

With the 20-colony MIC end-point, the mean sensitivity to PAS of the Indian strains was 2.13 γ per ml. and that of the British strains was 0.83, a difference significant at the 1 per cent level. However, there was no significant difference with the 50-colony or the 100-colony MIC. Thus, in this study as in the other two, Indian strains on the average were less sensitive to PAS than British strains.

Discussion

A detectable difference in the survivor curves for the Indian and the British strains apparently occurs only if the concentration of PAS is sufficiently high to allow less than 50 colonies to grow. A small proportion of the bacilli in Indian strains is apparently capable of growth in a medium containing high PAS concentration, while in the British strains there are few, if any, such bacilli. The presence of this resistant "tail" accounts for the difference in the average sensitivities of Indian and British strains at the 20-colony end point.

Apart from the resistant tail, the organisms in the Indian and British strains appeared to have a closely similar pattern of sensitivity to PAS, with no differences at the 50- and 100-colony end points.

Despite the difference in average sensitivity of Indian and British strains at the 20-colony end point, the variation from patient to patient was similar for Indian and for British strains. It may, therefore, be concluded that the presence of the resistant tail, which was responsible for the apparently increased resistance of the Indian strains, is a general property of these strains and is not merely a characteristic of the organisms from a few individual Indian patients. In other words, a finding of resistance, using the 20-colony end point, in a strain from an Indian patient is not likely to indicate that this particular patient is harboring a large proportion of resistant organisms, but only that in the particular test some factor, such as a chance large inoculum, was responsible for demonstrating the small proportion naturally present in all Indian strains. For similar reasons, little relationship would be expected between pretreatment PAS resistance (again using the 20-colony end point) and the subsequent ap-

pearance of resistance to isoniazid among Indian patients treated with isoniazid and PAS. PAS-resistant strains, on the same criterion, would be expected to occur sporadically during the early months of treatment.

Thus, definitions of resistance based on the 100-colony end point with an inoculum of about 10^5 viable units appear to be more reliable than on the 20-colony end point and to be less affected by the inoculum size. However, decreasing the inoculum size should have an equivalent effect when the end point is read at 20 colonies. Therefore, it is proposed to make the standard inoculum contain approximately 10^4 organisms in future work with Indian patients and to continue to use 20-colony end point resistance ratios.

A Study of Mycobacteria Isolated from Cervical Lymph Glands of African Patients in Kenya

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The isolation and typing of mycobacteria from tuberculous cervical lymph glands of African patients was undertaken jointly by the World Health Organization and members of the Kenya Medical Department as a corollary to a tuberculosis prevalence survey and a tuberculosis control project started in Kenya in 1958. Since the source of infection would have a bearing on control methods, it was important to know whether a significant number of infections was due to bovine or unclassified mycobacteria.

Lymph gland specimens collected from patients throughout Kenya who had not received antituberculosis drugs were shipped by air to the Tuberculosis Institute in Prague where they were examined bacteriologically and histologically. Precautions were taken to keep the specimens on ice as far as possible in shipping and to protect them from light.

In Prague, cultures were made from the specimens, the strains were typed and were tested for pathogenicity by growth pattern and guinea pig inoculation, and for drug susceptibility.

Laboratory methods: All strains showing typical eugonic growth in culture, virulence in guinea pigs, and a usual drug-susceptibility pattern were typed as *Mycobacterium tuberculosis* var. *hominis* (32 strains). Strains showing dysgonic growth were tested in the rabbit and mouse as well as in the guinea pig (9 strains). If the culture was negative but primary guinea pig inoculation was positive, the strain was isolated from the animal for further tests.

Of 57 specimens, *M. tuberculosis* was isolated by primary culture and/or guinea pig inoculation in 47 instances. In 41 of these, the strain was grown in subculture. All but one of the 41 strains were fully virulent for guinea pigs and all showed characteristic cord formation and were catalase positive. There was no striking evidence of drug-resistant organisms. Granulation tissue was found in 7 patients from whom acid-fast bacilli were not isolated. Three specimens were completely negative.

Clinical picture: Half of the tuberculous specimens were excised from children believed to have been less than ten-years old, and 19 per cent from patients aged ten to twenty years, with slightly more than 30 per cent from adults.

Twice as many of the glands were from the left side of the neck as from the right. There were three instances of bilateral involvement. Microscopic examination of sputum specimens from 49 of the patients gave only one positive result.

Chest films were obtained for 40 patients. The films, read in Nairobi, showed active pulmonary disease in 20, no abnormality in 16, and pulmonary calcification and pleural reaction in 2 each.

Discussion

All strains isolated appeared to be human strains of *M. tuberculosis*. No bovine or unclassified strains were obtained, which is somewhat surprising in view of the high proportion of glandular tuberculosis among Kenya Africans. Much more extrapulmonary tuberculosis is reported from Africa than from Europe or America, and it might be expected that there would be a relatively high prevalence of bovine infection or that unclassified acid-fast bacilli would be found. However, infection in cattle seems to occur in well-defined areas or pockets in Africa. In Kenya, veterinary officers report very little tuberculosis in cattle.

A correlation could be seen between the clinical evidence and the absence of bovine infection. There were no primary lesions on tonsils, but there was a high proportion of pulmonary lesions among the patients, suggesting that the cervical lymph node involvement is a hematogenous and lymphogenous dissemination from lesions in the lungs or hilar lymph nodes. Some workers in Africa regard tuberculous glandular involvement as a mark of failure to localize and circumscribe a primary

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focus rather than as a primary infection in itself. The material here discussed would seem to support these views. Further, the absence of bovine infection is in accord with the views of the African veterinarians who have regarded tuberculosis among cattle as a rarity and risk of human infection from that source slight.

The cultural, pathogenic, biochemical, and other characteristics of the strains show that mycobacteria isolated from cervical nodes of African patients do not differ in any essential respect from human strains similarly isolated from European patients.

By Title

Effects of Storage and Transport on the Cultivability of Mycobacteria. LADISLAV ŠULA, T. K. SUNDARESAN, AND M. LANGEROVÁ.

A Comparison of the Sensitivity of Löwenstein-Jensen Media Prepared in Different National Bacteriological Laboratories. LADISLAV ŠULA, T. K. SUNDARESAN, AND M. LANGEROVÁ.

Comparison between Attenders and Non-Attenders at the Danish Mass Tuberculosis Campaign, 1950–52. Tuberculosis Morbidity and General Mortality in the Two Groups during the First Four Years of Follow-up. OLE HORWITZ AND JORGEN KNUDSEN.

A Comparison of Purified Tuberculins in the South-Eastern USA. G. W. COMSTOCK.

Simultaneous Multiple Tuberculin Testing. SOL ROY ROSENTHAL AND J. E. P. LIBBY.

Correspondence

"HISTOPLASMOSIS—A 'SPELUNKER'S' RISK"

To the Editor of the American Review of Respiratory Diseases:

In his case report entitled "Histoplasmosis—a 'Spelunker's' Risk" which appeared in the February, 1961, issue of the AMERICAN REVIEW OF RESPIRATORY DISEASES, the author, Dr. Jackson, makes the following statement: "The soil is the natural reservoir of this saprophyte [*Histoplasma capsulatum*], and man acquires the infection as a result of inhaling air-borne spores. *It is also quite clear that the soil becomes contaminated by the spore-laden droppings of birds and bats* (1)." (The italics are mine.) The reference given by the author is to a paper by Larsh and associates (Amer J Hyg, 1956, *63*, 18).

The first of the two sentences quoted above would be generally acceptable to most, if not all, of the authorities on histoplasmosis. The italicized sentence, however, not only contradicts the first, but has two errors that are representative of the existing confusion in many quarters concerning the relationship of birds and histoplasmosis. Because of the frequency with which *H. capsulatum* has been isolated from soils containing the droppings of chickens and other birds, it has been assumed by many that the birds are infected and contaminate the soil with their excreta. No evidence has ever been produced to indicate that this sequence of events actually occurs. As a matter of fact, it has been shown experimentally by Ajello, and substantiated by others, that chickens do not become diseased even after massive inoculations of the fungus intraperitoneally. The organisms are apparently destroyed by the chickens because it is subsequently impossible to recover them on culture of the abdominal viscera. The explanation of this experimental finding probably lies in the fact that *in vitro* the fungus will not grow at 42°C., the normal body temperature of the chicken. This too was demonstrated by Ajello.

Larsh's paper, to which Jackson has referred, contains no statement that could be interpreted as indicating that birds become infected with, or are a reservoir of, the fungus. On page 26 Larsh states: "It is important to note that *H. capsulatum* has not been isolated from chickens. In our laboratory a series of these animals was inoculated intranasally and intraperitoneally with the fungus. In no instance was the organism isolated from experimental animals.... Feces from these chickens processed prior and subsequent to inoculation failed to yield the fungus. From these and other experiments" (Larsh here refers to prior work in this field done by the present writer and his associates, Amer J Public Health, 1952, *42*, 930) "it is evident that chickens do not harbor the organism." Similar opinions have been expressed by such authorities as Emmons (pp. 79, 80), Ajello (p. 91), and Furcolow (p. 130) in *Histoplasmosis*, edited by Henry C. Sweaney (Charles C Thomas, publisher, 1960).

The second error in Jackson's statement concerns "spore-laden droppings of birds and bats." As noted above, droppings of birds have never been shown to harbor *H. capsulatum*. The droppings of infected bats may contain the fungus, since mammals can and do become infected. The fungus in the excreta would not be in the spore form, however, but would be in the yeast phase, so that at best, or at worst, the droppings would be "yeast-laden."

The explanation for the association between bird droppings and *H. capsulatum* in soil remains obscure. It is likely that the droppings possess some property that makes soil a better culture medium for the fungus. To say that birds infect the soil is a clear example of "guilt by association," which is considered reprehensible in political circles, and has no place in science except as a lead for further investigation. It might be well to correct these errors before the poultry industry and the Audubon society sever relations with the American Thoracic Society.

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March 15, 1961

Books

AN OUTLINE OF CHEMICAL GENETICS. By Bernard S. Strauss. Pp. 188, W. B. Saunders Company, Philadelphia, London, 1960. Price, $5.00.

There is an increasing need for small authoritative monographs on specialized subjects which summarize information that is scattered widely throughout the scientific literature. Such monographs are not easily written because the author must simplify without loss of precision. Dr. Strauss has written a pleasant and informative book which successfully summarizes much of our current knowledge on chemical genetics. Although the author is naturally at his best when discussing his own area of professional competence, his pen flows easily throughout most of the book. The monograph had its origin in a series of lectures to the Faculty of Medicine at Osaka University, and this event in itself is evidence of the growing conviction that, although many of the problems of human genetics are unique, the human geneticist is well advised to acquaint himself with relevant work in other organisms. The universal principles of biology and the uniqueness of man are not contradictory.

Following interesting chapters on the historical development of biochemical genetics and the chemical nature of the hereditary material, the author discusses the central problem of the relationship between genes and protein synthesis. The treatment of genetic recombination at the molecular level is clearly and simply discussed in the next chapter. The discussion of chemical mutagenesis which follows is particularly timely, for there is growing concern that the exposure of man to various chemical mutagens may be as important as his exposure to ionizing radiation. A chapter on nucleocytoplasmic relationships and the problem of protein synthesis is a welcome surprise, for this important aspect of protein synthesis is frequently neglected. The illuminating experiments of Briggs and King on the role of the nucleus in differentiation are properly emphasized. The brief chapter on human biochemical genetics emphasizes examples of diseases in man caused by simple gene substitutions. It is probably inevitable that much of the recent work on the abnormal hemoglobins could not be included, for this is an area in which research is proceeding at a great pace. The discussion of the Hardy-Weinberg equilibrium is too short to be useful, and would have been better omitted or presented in more detail.

In general, the book can be recommended for what it is, an outline of chemical genetics. It forms a good companion to the recent book by Harris on human biochemical genetics. Dr. Strauss's monograph is well produced and the format is clear. It can be read with profit and pleasure by both microbiologists and physicians concerned with the growing role of genetics in modern medicine.

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CONGENITAL MALFORMATIONS. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecelia M. O'Connor. Pp. 308, Little, Brown and Company, Boston, 1960. Price, $9.50.

The Ciba Symposium on Congenital Malformations was held in January of 1960 as an international and interdisciplinary forum for the consideration of the causes of teratogenesis in the hope that it might stimulate "a world-wide effort to eliminate many of these avoidable calamities." Chaired by Professor Hamilton, an anatomist, embryologist, and medical school dean, the meeting was comprised of contributions and informal discussions from authorities in the fields of anatomy, atomic energy research, embryology, genetics, medicine, obstetrics and gynecology, pathology, pediatrics, pharmacology, physiology, and public health and preventive medicine. Through the publication of this book, the reader may share in the papers and discussions of this stimulating symposium.

McKeown and Record emphasized the importance of observing malformations in a population, not only at birth, but for five years after birth. They reported on the preliminary results of a continuing prospective study in which records were completed for all births in Birmingham within two weeks after birth and for five years after birth, noting the incidence of anomalies and the association of multiple malformations as well as their occurrence in multiple births.

Penrose concisely summarized new information on genetic causes of malformations and stated that the differentiation between environmental and genetic causes of a developmental anomaly is becoming increasingly difficult, since it is known that environment does not merely regulate the manifestations of genes, but can affect the genes themselves and their arrangement on chromosomes.

Ford reviewed the discoveries within the pre-

ceding twelve months of five types of chromosomal abnormalities in association with congenital malformation and described two further examples, one involving an error in the number of autosomes and the other a structural change associated with a mongol phenotype.

Ingalls discussed the role of environmental factors, especially rubella, hypoxia, and influenza in human pregnancy, and stressed the importance of the time of injury with relation to fetal age and development.

Murphy studied the effect of eight tumor-inhibiting chemicals on the pregnant rat and its fetuses, and described the fetal abnormalities produced by introduction of the agents on the twelfth day of gestation.

Tuchmann-Duplessis and Mercier-Parot followed with a report on a drug, actinomycin D, which induced malformations of most of the body systems of the rat when injected in large doses (four to five times the therapeutic dose) on the seventh to ninth days of gestation.

Nelson reported similar effects on embryonic tissue from a deficiency of a vitamin, pteroylglutamic acid.

Woollam and Millen turned their attention to the possibility of modifying the deleterious action on the embryo of certain agents, hypervitaminosis A and X-irradiation, by giving hormones such as thyroxine, insulin, and cortisone. The expected effects of the teratogenic agent could be altered, but the authors cautioned conservatism in interpretation of the interaction of agents.

McLaren and Michie changed the discussion to one of "congenital runts," animals stunted at birth beyond the extreme variation of normal distribution, possibly because of limited maternal blood supply.

Various defects in closure of the encephalic tube, and their possible genetic and environmental causes, were discussed by Giroud.

Hoet and his associates directed their attention to the role of the mother's health, especially as it concerns endocrinopathies, and expressed the hope that reproductive wastage and malformations might be prevented by a prenatal evaluation of the mother and by treatment of those with mild endocrine disturbances.

Stevenson reported that hydramnios is the most important single complication of pregnancy associated with fetal malformation and death, and he decried the lack of information in general about the pathogenesis and incidence of hydramnios.

A general discussion followed, with many unanswered questions that may well be the subject of a future interdisciplinary symposium on congenital malformations.

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Fundamentals of Chest Roentgenology. By Benjamin Felson. Pp. 301, W. B. Saunders Company, Philadelphia and London, 1960. Price, $10.00.

Benjamin Felson is an astute clinician, a thoughtful radiologist, and a considerate teacher with a good sense of humor. These assets have resulted in an instructive, enjoyable, but erudite book on chest roentgenology which can easily be read "cover to cover," but which will also be useful for consultation and reference.

The keynote of this work is the emphasis on analysis and fundamental radiographic concepts as the building blocks for roentgen diagnosis. Thus, the roentgenographic changes are discussed in terms of physical diagnosis instead of disease entities.

There is a casual introductory discussion of the roentgenographic examination which indicates the radiologic methods that can be employed to clarify the obscure or hidden densities of the chest film. Fluoroscopy is evaluated as a method requiring skill, care, and knowledge to ensure that its benefits far outweigh its possible hazards. Specialized and angiographic techniques are not discussed in detail.

A subsequent chapter on localization of intrathoracic lesions emphasizes the importance of roentgenographic analysis, with special attention to the silhouette sign and the air bronchogram.

Chapters are devoted to the lobes, segments, hili, vessels, lymph nodes, and the pleura. Variations in normal anatomy are described in detail. The confusing variations in segmental bronchial anatomy are well summarized and illustrated.

The final chapters are devoted to the extrapleural space, the diaphragm, special roentgenographic signs and their significance, and a review of 18,000 normal chest roentgenograms.

The illustrations are well chosen and reproduce well. This book should be of great value to all concerned with the diagnosis of diseases of the chest. It is particularly recommended to students in the fields of radiology and chest diseases as a fundamental approach to the art and science of chest roentgenography.

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A Biochemical Approach to Pathology. By M. J. R. Dawkins and K. R. Rees. Pp. 128, Butler and Tanner Ltd., Frome, and London, 1959. Price, $4.50.

The ambit of this pocket-size book is introduced by a concise outline of pertinent information about the biochemistry of normal intracellular metabolism. The authors then set about to succinctly delineate the effects upon these metabolic processes of a multitude of exogenous, endogenous, and bacterial toxins; nutritional deficiencies; and inborn genetic errors. A final chapter treats the biochemical mechanisms of cellular growth, differentiation, and anaplasia.

To accomplish so formidable a task, the schisms that exist in theoretic matters are largely bypassed or hazardously bridged, and the chasms of uncertainty and doubt in practical knowledge are often skirted or disregarded in abbreviation and oversimplification. Although few errors are to be found in this scholarly work, minor transgressions of precise knowledge have arisen out of efforts at clarification. These are made more obscure by the presence of only an abbreviated, general bibliography.

Generally, the text is clear, concise, and authoritative. It provides an animated review of current thought and knowledge in the conjoint fields of biochemistry and pathology. Although it cannot adequately serve as a reference text in the absence of a full bibliography nor, for long, persist as a source of current factual information in these rapidly advancing fields, the book can be strongly recommended to the biochemist with curiosity about the morphologic aspects of disease and to the pathologist who is inquisitive about the fundamental biochemical derangements that underly cytologic alterations.

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Infectious Diseases of Children. Second Edition. By Saul Krugman and Robert Ward. Pp. 398, The C. V. Mosby Company, St. Louis, 1960. Price, $13.00.

Published just two years after the first edition, the second edition of this book reflects primarily the rapid advances of knowledge in the field of clinical virology. This accumulation of new data, accompanied as it was by abrupt changes in nomenclature, has resulted in considerable confusion among physicians about the relative importance of various viruses in clinical practice and the relationship of a certain agent to a particular syndrome.

This book will serve as an excellent, clear and concise guide through this jungle. The material is remarkably up to date, and is organized to provide the maximal amount of useful information to the practitioner. New in this edition is a chapter on acute respiratory infections which should prove particularly valuable; a chapter on rabies has been added which stresses the practical problems encountered in the attempted prophylaxis of the disease. Numerous other sections of the book have been extensively revised, particularly the chapter on enteroviruses, which includes a new discussion of the clinical syndromes associated with various ECHO viruses, and additional material on live, attenuated poliomyelitis vaccine.

As in the previous edition, the authors do not attempt to present a comprehensive coverage of all infectious diseases of children, but limit themselves more or less to those encountered in the temperate zones of the western world. There is no discussion, for example, of the parasitic diseases, except those due to toxoplasma. However, within this volume, the physician, with the help of the excellent index, will be able to discover the answers—if such exist—to many questions which may arise during his every-day practice.

This book can be recommended as an essential item in the library of all medical and public health personnel involved in the care of children. It will also prove of great interest to any individual who would like to keep abreast of the rapid developments in the field of infectious diseases.

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American Thoracic Society

Medical Section of the National Tuberculosis Association

The Award of the Trudeau Medal for 1961

The Trudeau Medal for 1961 was awarded to Dr. Selman A. Waksman at the annual meeting of the National Tuberculosis Association, held in Cincinnati, Ohio, May 21–25, 1961

The Presentation was made by Dr. Esmond R. Long

DR. LONG: The recipient of the Trudeau Medal of 1961 is a man of great distinction in American medical science. He has a quite special relationship to the National Tuberculosis Association. Just fifty years ago he entered the university on which he was ultimately to confer unusual fame. Almost immediately he began what soon became his life endeavor, the study of microorganisms in the soil and their relation to each other and to the science of agriculture. By 1930 he was among America's greatest experts in this broad field.

In 1932, the Chairman of the Division of Medical Sciences of the National Research Council, who was also Chairman of the National Tuberculosis Association's Committee on Medical Research, persuaded him to undertake experiments on a special phase within that field, viz., the fate of tubercle bacilli in competition with other bacteria in the soil. In order to speed the prosecution of that research the Association furnished him with a National Tuberculosis Association Fellow as special assistant. Our medallist's investigations with this assistance, and to an even greater extent independently of it, soon led to the identification of fungi in the soil with a strong inhibitory action on many bacteria.

Several of these fungi were discovered; we are concerned chiefly with just one of them. In 1943, in association with a group of able assistants, tonight's recipient of the Trudeau Medal discovered the highly potent antimicrobial, streptomycin, in a soil fungus. It is not necessary to remind this audience of the significance of that event. It revolutionized the medical treatment of tuberculosis and marked the first big break-through in the age-old attack on this disease. The spectacular successes of more recent years all stem back to this pioneer discovery.

It is a privilege and honor, therefore, for us this year, the semicentennial of our medallist's commencement of his work at Rutgers College, to confer the Trudeau Medal on this distinguished investigator, Director Emeritus of the Institute of Microbiology of Rutgers University, President of the internationally active Foundation for Microbiology, recipient of awards, prizes, and honors from all the world, Doctor Selman A. Waksman.

DR. WAKSMAN: It is indeed a great honor that you have bestowed upon me tonight. There is no greater name attached to the care and treatment of tuberculosis sufferers, at least in this country, than that of Edward Livingston Trudeau. Upon the statue of this pioneer, which stands upon the old Fox Run facing the Adirondack Mountains to the east, there is engraved the following:

> Cuérir Quelquefois,
> Soulager Souvent,
> Consoler Toujours.

This can be broadly translated to mean: "To Cure Occasionally, To Relieve Often, and To Console Always."

Dr. Trudeau's son, Francis B. Trudeau, wrote, in December, 1943, in the preface to the Fourth Edition of his father's autobiography:

> Though the specific cure for tuberculosis still eludes us, the good work goes on ... the two major interests of his life, the sanatorium care for the tuberculous, and the scientific research into the origin and nature of the disease, which had such modest beginnings in the 'Little Red' and the homemade laboratory, have developed in ways undreamed of a generation ago.

Think only that this was written about three months after we had isolated, in a modest soil microbiological laboratory, but not yet announced, the organism that produces streptomycin. This was soon followed by a second report on the effect of streptomycin upon the tuberculosis organism, namely, in February, 1944. Within less than a week after the appearance of this paper, Dr. Corwin Hinshaw and Dr. William Feldman of the Mayo Clinic came to see me in my laboratory in order to arrange for cooperative work on the treatment of experimental tuberculosis with streptomycin, then in clinical therapy. November 20, 1944, is an important historical date. On that day, streptomycin

was first administered to a human being. The patient in question was a twenty-one-year-old white girl who had progressive, far advanced pulmonary tuberculosis.

The rest is history. It is these two pioneers, Drs. Hinshaw and Feldman, who must be recognized as having first demonstrated the clinical effectiveness not only of streptomycin but of any drug in the treatment of the Great White Plague, tuberculosis.

My time is too limited to trace even briefly the subsequent developments. Streptomycin, para-aminosalicylic acid and, still later, isoniazid have all served to deliver a death blow to the various forms of this disease. I can do no better than cite from a recent report by Dr. G. J. Drolet of the New York Tuberculosis and Health Association who, speaking of child mortality, said:

> In New York City, the number of deaths fell between 1950 and 1959 from 78 to 14, or by 82 per cent, when the child population was rising by 233,703. In 1930, these deaths numbered 400. For the United States as a whole, during the period 1950 to 1959, the deaths fell from 1,531 to 230, or by 85 per cent, even while the number of children under fifteen rose from 40,000,000 to 55,000,000. In England and Wales these deaths decreased by 92 per cent. In the Scandinavian countries the decline was also strikingly high: 86 per cent in Denmark, and 95 per cent in Sweden; in Norway, not one death.

Who could have dreamed of this only fifteen or sixteen years earlier? I am told, on good authority, that prior to the advent of streptomycin, there were on record throughout human history altogether only 50 cases of spontaneous recovery from tuberculous meningitis. Even some of those 50 may not have been properly diagnosed.

Who could describe the anxiety of the parents of those children or their happiness upon seeing their dear ones return to life from certain death. In those early days soon after the discovery of streptomycin, when little was known of its purity and of its effects upon the various human organs, many a father, who had been forewarned of the possible undesirable reactions, said to me, "I would rather have a deaf child than a dead child."

The future looks bright indeed. May I be permitted to quote further from an address by Dr. J. L. Wilson, delivered in 1954, before the Pennsylvania State Health Conference.

> One hundred years hence, tuberculosis will probably be as much a rarity as leprosy is today. Medical students and physicians taking postgraduate courses will learn the textbook picture of tuberculosis and be told that they must always be on the watch for cases arising in endemic foci in certain backward parts of the country or flown in from remote places of the shrunken globe. Medical men who are interested will have to fly to the peninsula of upper Michigan (or to Asia) if they wish to study the manifestations of tuberculosis, because there the United States Public Health Service operates the only remaining American sanatorium.

In order to accomplish this great dream, however, all the efforts of the American Thoracic Society and of its parent organization, the National Tuberculosis Association, are required. I am sure that those who are assembled here tonight will make this effort. I can only express my own feeling in the hope that this will be accomplished.

The Use of Chemotherapy as a Public Health Measure in Tuberculosis

A Statement by the Committee on Therapy

In November, 1959, the Arden House Conference on Tuberculosis recommended the use of adequate chemotherapy as the major tool for the eradication of tuberculosis in the United States. For background on the need for a wider use of chemotherapy as a public health measure, reference should be made to two recent editorials (1, 2). The full implementation of this proposal would entail a great increase in the number of persons receiving chemotherapy. For this reason this statement is offered to define adequate chemotherapy for the individual as a public health measure in order to ensure the proper use of this tool. It is intended to serve three purposes:

1. To define groups of individuals in whom the potential for exacerbation and progressive tuberculosis constitutes a risk to individual and to the public
2. To define the minimal standards of chemotherapy for nonhospitalized patients
3. To offer these recommendations as a guide to all physicians who may encounter individual cases of tuberculosis as well as to those public health officers and physicians directly responsible for the implementation of chemotherapy in outpatients

It is to be emphasized that this statement is not intended as a detailed outline for the management of the patient with tuberculosis.

The Committee on Therapy has previously issued statements on standards for the home care of patients with tuberculosis (3). The definition of a program for the adequate treatment of tuberculous patients outside the hospital must not be construed to imply that *outpatient treatment* is recommended as a substitute for hospital care of patients with active tuberculosis. Any patient who is excreting tubercle bacilli in the sputum should be hospitalized. In the unusual instance in which this is impossible, it is still of particular importance that drug susceptibilities of the organisms be determined and followed as long as tubercle bacilli can be cultured from the patient. The presence of drug-resistant organisms requires that the patient be hospitalized for thorough evaluation under conditions in which the most precise control is available. It is hoped that this statement may help in the selection of those persons who should receive chemotherapy for tuberculosis but who for various reasons are not hospitalized.

Minimal standards of drug treatment for nonhospitalized patients: Isoniazid is the most potent drug presently available against tubercle bacilli. Whenever antituberculosis chemotherapy is indicated the patient should receive isoniazid in a minimal dose of 5 mg. per kg. of body weight daily. The drug may be given in 2 or 3 divided doses. The minimal duration of continuous treatment with isoniazid should be 12 months, but in most instances it should be continued for 18–24 months or for at least 6 months beyond all evidence of activity of the tuberculous process. In selected patients the period of chemotherapy may be considerably prolonged, even to the point of continuing the suppression of the disease for life in some patients.

Whenever initial antituberculosis chemotherapy is being given for overtly active (bacteriologically positive) tuberculosis, a companion drug should be given concurrently. The most commonly used second drug is para-aminosalicylic acid (PAS) which may be given in doses of 10–12 gm. per day in divided doses of the standard product or of 6 gm. per day of the combination with ascorbic acid (PAS-C). If serious drug toxicity or hypersensitivity develops, the patient should be admitted to a hospital for evaluation of the status of his disease and an appropriate regimen devised.

Isoniazid may be used alone in certain circumstances. In most of the patients who would fall into Categories II and III below, such chemotherapy would be adequate. These categories comprise patients from whom tubercle bacilli are being excreted in very small numbers, if at all, and in whom the therapy is being used to prevent complications (as with a recent asymptomatic conversion of the tuberculin reaction (4, 5)), or for long-term suppressive therapy to prevent reactivation of disease.

All patients treated on an outpatient basis should be followed as closely as possible by the health team. Physical examination and appropriate laboratory and roentgenologic follow-up studies should be made monthly at the outset of therapy. The period may be lengthened later to 3 or 4 months, depending upon the individual patient and the local facilities and personnel available. Careful questioning of the patient regarding the fidelity with which he follows the prescribed drug regimen should be supplemented by the use of the available urine tests for isoniazid (6) or PAS (7).

Unless this minimal program can be maintained, the risk of inadequate treatment can outweigh the benefits of chemotherapy. Public health and clinic nurses may be helpful in ensuring adequate and continuous chemotherapy. It cannot be overemphasized that the patient treated outside the hospital must be closely supervised.

Categories of patients for whom chemotherapy is indicated:

I. Mandatory. In the following instances chemotherapy of tuberculosis is mandatory:
   1. Patients who have been discharged from the hospital or sanatorium with or without medical approval and who have not completed their full course of chemotherapy.
   2. Exceptional cases of clinically active tuberculosis in which hospitalization of the patient is not feasible.

II. Strongly indicated. In the following circumstances, there is good evidence that chemotherapy for tuberculosis should be given to prevent progression of the disease:
   1. Tuberculin-positive children less than 3 years of age. Because of their youth the patients must be presumed to have active disease, even in the absence of any clinical evidence of illness.
   2. Patients with previously diagnosed tuberculosis in whom activity is uncertain or shown only by minor changes demonstrable on serial roentgenograms even in the *absence* of symptoms and bacteriologic evidence. A substantial number of persons would fall into this category if serial roentgenograms were scrutinized and more frequent bacteriologic studies were made on persons now carried as "inactive" on the tuberculosis registers. Such persons constitute one of the largest unrecognized reservoirs of tuberculosis in the population today and a group with which the Arden House Conferees were particularly concerned.
   3. Tuberculin-positive persons who have disease or syndromes for which they are receiving prolonged corticosteroid therapy.
   4. Tuberculin-positive individuals with inactive pulmonary lesions by roentgenographic criteria who are to undergo, or who have had, gastric resection.
   5. Unstable or severe diabetics who are tuberculin positive.
   6. Patients with nodular silicosis who have positive tuberculin reactions.

It should be mentioned that the magnitude of the reaction to tuberculin has a bearing upon the indications for chemoprophylaxis in this group. Thus, a reaction of 20 mm. induration to intermediate-strength tuberculin (5 tuberculin units) constitutes a generally more urgent indication than a reaction of 10 mm. to second-strength tuberculin (250 tuberculin units).

III. Discretionary. In addition to the indications for which there is good evidence of the need for chemotherapy, it may be used in the following situations at the discretion of the physician. The magnitude of the tuberculin reaction should enter into the decision in this group also.
   1. Household contacts of patients with sputum positive for tubercle bacilli regardless of age. The indications in those with positive tuberculin reactions and under the age of 3 are covered in Category II.
   2. Children of less than 14 years with positive tuberculin reactions (5).
   3. Persons with positive tuberculin reactions and pulmonary lesions for whom careful diagnostic study has failed to reveal evidence of another disease. Tuberculosis accounts for a high proportion of such indeterminant lesions, and early therapy is advisable if other diseases (particularly carcinoma) have been excluded.
   4. Children with positive tuberculin reactions who have severe viral infections, especially measles.
   5. Pregnancy in a woman with inactive tuberculosis. Therapy may be started in the last trimester and should be continued for several months after delivery.
   6. A definite conversion from a negative to a positive reaction to 5 TU of tuberculin (intermediate PPD) in an adult (5).

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The Use of Antimicrobials in Viral Infections of the Lung

A Joint Statement of the Committee on Therapy and the Committee on Respiratory Diseases in Children

The antimicrobials so effective in many bacterial infections are unfortunately prescribed too frequently for viral infections of the respiratory system. Bacterial complications of viral pneumonias are uncommon, and there is no evidence that the currently available antimicrobials will favorably influence human pulmonary viral infections except for those caused by certain of the larger filterable agents. Chemotherapy specifically effective against viruses is handicapped by the very close similarity between the metabolic and biochemical processes of the virus and those of the host cell. While considerable progress is being made in the selective inhibition of intracellular virus reproduction by chemical agents (1), work in this field has not yet reached the stage of effective therapy of human diseases.

This statement will attempt to define the place for the commonly used antimicrobials in viral pulmonary infections and those presumed to be of viral origin. Problems in diagnosing viral pneumonias are often such that the physician may be uncertain of the nature of the infection at the time when a decision about therapy must be made. A detailed discussion of differential diagnosis is beyond the scope of this statement. The recommendations made here are applicable to those situations in which the diagnosis of a viral infection is reasonably certain or to those in which clinical, epidemiologic, and laboratory studies lead to a presumptive diagnosis of a viral pulmonary infection.

Five groups of diseases will be considered: (*1*) psittacosis-ornithosis, (*2*) primary atypical pneumonia, (*3*) influenza, (*4*) pneumonias due to the viruses of varicella and rubeola.

(*1*) *Psittacosis-ornithosis:* Psittacosis is a rather uncommon disease caused by a filterable agent of large size. Clinically this disease varies in severity from a mild respiratory infection to a severe and at times fatal pneumonia. There are no clinical features that clearly distinguish it from other viral pneumonias, but a history of exposure to birds (especially psittacine birds or domestic fowl) should suggest the possibility of this disease. The diagnosis can usually be confirmed by increase in titers of complement-fixing antibodies as demonstrated in serum specimens collected two weeks apart. Although a number of antibacterial agents favorably influence the disease in experimental animals, only the broad-spectrum antimicrobials appear to have significant effect on human infections. No conclusive clinical studies have been reported, but good experimental evidence is available suggesting that tetracycline is superior to chloramphenicol.

Tetracycline therapy is recommended for psittacosis. Two grams per day are usually adequate in adults, although in seriously ill patients 3 gm. per day may be used for a few days. In children a dosage schedule of 10–20 mg. per pound of body weight per day is recommended. Early treatment may interfere with the development of antibodies, and it has been observed that short courses of treatment may be followed by relapse. Treatment should be continued for at least one week after the patient becomes afebrile.

(*2*) *Primary atypical pneumonia:* Present evidence indicates that there is probably more than one etiologic agent responsible for primary atypical pneumonia. This syndrome has been diagnosed on the basis of certain characteristic clinical features, on the exclusion of bacterial pneumonias, tuberculosis, psittacosis, influenza, et cetera, and on the demonstration in some instances of cold agglutinins or Streptococcus MG agglutinins. There is convincing evidence that the agent first described by Eaton and associates (2) in 1944 is one of the causes of primary atypical pneumonia. The exact nature of the Eaton agent has not been de-

termined. It is in the category of the larger filterable agents and is clearly distinguishable from the virus of psittacosis.

Since primary atypical pneumonia is ordinarily benign and resolves spontaneously, an improvement in the clinical situation or in the chest roentgenogram which follows initiation of therapy cannot be clearly attributed to therapy. The few well-controlled clinical studies of the use of antimicrobials in primary atypical pneumonia have yielded conflicting results (3, 4), possibly because more than one agent may produce this clinical syndrome. Kingston and co-workers (5), in a recent study, showed that demethylchlortetracycline favorably altered the course of Eaton agent pneumonias.

The place for antimicrobials in primary atypical pneumonia is not entirely established and the mild nature of many of the cases makes the routine use of antimicrobials unwarranted. However, when the disease is severe and is associated with high fever, the use of tetracycline may be beneficial. If tetracycline is used, a dosage of 2 gm. per day in adults and 10–20 mg. per pound per day in children is recommended. Treatment should be continued for five to seven days.

(3) *Influenza:* Influenza, whether occurring in pandemics, epidemics, or in localized outbreaks, has a rather uniform clinical pattern though varying considerably in severity. Nevertheless, in the absence of a well established outbreak, serologic evidence of influenza, or isolation of the virus, it is not possible to establish the diagnosis with certainty. Undoubtedly, numerous other respiratory infections are mistaken for true virus influenza. All studies, both in animals and in human beings, have failed to demonstrate an effect on the influenza virus by any of the currently available antimicrobials.

Bacterial complications are not common in influenza; but at times, such as in the 1957 Asian influenza epidemic, superimposed bacterial infections may be severe and on occasion rapidly fatal. Nevertheless, the routine use of antimicrobials as prophylaxis against bacterial infections is clearly not indicated. The unwarranted use of antimicrobials in outbreaks of influenza actually may be responsible for an increase in troublesome infections with certain antimicrobial-resistant organisms. Antimicrobials therefore should be withheld unless there is good evidence of complicating bacterial infections. Every effort should be made to isolate the secondary bacterial agent and to determine its susceptibility to antimicrobials.

(4) *Other viral infections of the lung:* In recent years a number of small or "true" viruses have been isolated from cases of respiratory infection and have become established as primary etiologic agents of viral pneumonia. The adenoviruses have been isolated from cases of viral pneumonia, especially in service recruits. The parainfluenza viruses (myxoviruses other than influenza viruses) and the respiratory syncytial virus have been isolated from patients with pneumonia, especially children. Recently a few cases of severe pneumonia have been reported which seem to be due to certain of the Group A Coxsackie viruses. Pneumonia resulting from ECHO viruses is unusual, but cases have been reported.

There is no evidence of beneficial effect from antimicrobials in uncomplicated viral infections in this group. The physician should always be on the alert for bacterial complications in these viral infections, but bacterial complications are not common enough to warrant routine use of antimicrobials.

(5) *Pneumonias due to varicella and rubeola viruses:* In some instances severe pneumonia may be caused by the viruses of chicken pox and measles. It is difficult to distinguish such infections from complicating bacterial pneumonias. When it has been shown to be primary uncomplicated varicella or rubeola pneumonia, antimicrobial therapy has not been of benefit. On the other hand, superimposed bacterial infections may be so hazardous in cases of measles that intensive antimicrobial therapy is advisable whenever bacterial infection is suspected.

Viral infections in chronic pulmonary diseases: In patients with chronic disabling pulmonary diseases, acute respiratory infections may lead to serious difficulties. Influenza has been found to be responsible for exacerbations in patients with emphysema, but little is known about the relationship between other specific respiratory viruses and exacerbations of chronic pulmonary disease. When there is reasonable doubt of the etiology of a respiratory infection in patients with chronic bronchitis, asthma, emphysema, or bronchiectasis, it is best to provide early adequate chemotherapy based on the results of sputum smears, cultures, and drug-susceptibility tests rather than to risk the consequences of untreated bacterial infection. The difficulties encountered in detecting bacterial infections in the presence of chronic bronchitis and the place for antimicrobial therapy in this situation were discussed in a recent statement of the Committee on Therapy (6).

Committee on Therapy

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(6) AMERICAN THORACIC SOCIETY: Bacteriologic considerations in the therapy of chronic suppurative bronchitis and bronchiectasis, A Statement by the Committee on Therapy, Amer Rev Resp Dis, 1960, *82*, 743.

Rest, Exercise, and Work in the Treatment of Tuberculosis

A Statement of the Committee on Therapy

For many decades rest was the keystone in the management of tuberculosis, but its therapeutic role has never been clearly defined. Prior to the advent of effective chemotherapy, the degree of rest prescribed in the initial phase of the management of a patient with tuberculosis varied from strict bed confinement to programs with moderate curtailment of usual activities. Few controlled studies comparing the influence of amounts and duration of rest were available before the chemotherapeutic era. While multiple adverse effects from strict bed rest are known to occur in healthy persons or in persons with other disease, little information has been obtained concerning the possible harmful physical and psychic effects of rest in patients with tuberculosis.

During the past few years a number of carefully controlled studies have indicated that rest plays only a minor therapeutic role in the treatment of tuberculous patients when an effective chemotherapeutic regimen is employed. Although the long-term results of these studies are not available and more investigation is necessary, the Committee believes that there is enough evidence and opinion available to warrant revising its statement concerning rest published in 1954.[1]

It is the Committee's opinion that patients who can be treated with an effective chemotherapeutic regimen should have their activity regulated as outlined in the seven categories which follow:

(*1*) *Bacteriologically positive with symptoms:* Patients with high fever, weakness, pain, productive cough, or dyspnea attributable to tuberculosis usually elect to rest in bed the greater part of the day. If the patient chooses extremes of rest or activity, the physician may be required to regulate the patient's activity more precisely. Irrespective of the rest employed, the patient should remain within the ward area. The time at which the patient may commence the use of the bedside chair, sitting up for meals, and bathroom privileges will be determined in a large part by the patient's physical ability to undertake these activities. Severe hemoptysis continues to be an indication for bed rest with the exception of the use of the bedside commode until the acute episode has safely subsided.

(*2*) *Bacteriologically positive with no symptoms:* Confinement of these patients to the ward area because of their infectiousness should not preclude the fullest possible utilization of occupational, educational, and recreational therapy, and physical reconditioning in preparation for their ultimate discharge and return to work. Full ambulation and ward activity should now be prescribed. It should be recognized that of itself confinement to the hospital results in less than the patient's usual amount of activity even though no limitation of activity is specifically ordered. Patients

[1] Bed rest in the treatment of tuberculosis, Amer Rev Tubere, 1954, *69*, 1069.

permitted full ambulation will often remain passive, if not in bed, for much of the day unless encouraged to activity by their physician.

(3) *Bacteriologically negative*[2] *with no symptoms:* At this point many patients, in whom surgery is not contemplated, may be discharged from the hospital to continue chemotherapy and be followed as outpatients. Further hospitalization may be necessary if the patient is considered unreliable, has unfavorable social and economic conditions, or would lack adequate outpatient facilities for follow-up care. For those who remain in the hospital, full activity should be recommended. Freedom should be granted to visit all areas of the hospital available to general hospital patients. Occasional visits outside the hospital with family or friends should be encouraged. If the patient has a cavity, it may be wise to limit his activity to the ward area for a longer period.

If thoracic surgery has been performed, discharge from the hospital should be recommended as soon as the following criteria have been met: (*a*) That postoperative cultures of sputum or gastric contents are negative for tubercle bacilli; (*b*) the residual disease is roentgenographically stable; (*c*) any complications have subsided; (*d*) adequate physical reconditioning has been accomplished; and (*e*) appropriate arrangements have been made for the continuation of chemotherapy and follow-up observation on an outpatient basis.

(4 *a*) *Post-hospital activities; bacteriologically negative and cavities closed:* If chemotherapy and ancillary measures such as surgery have achieved this successful result by the time the patient is discharged from the hospital, he may return promptly to his normal work and recreation within the limits of his general physical condition. If physical reconditioning has not been accomplished in the hospital, it should be attained as quickly as possible in the post-hospital phase. The patient should be encouraged to return to work as soon as possible even though he is receiving chemotherapy and may not be classified technically as having "inactive" disease according to the National Tuberculosis Association *Diagnostic Standards.*

Only rarely is it necessary to recommend a change in the patient's occupation because he has had tuberculosis. However, a change in occupation is recommended if he would be exposed to silicon dioxide dust. A change may also be indicated if the patient would be exposed to irritating dust and air pollutants. For those who must change occupations because of reduced pulmonary reserve or for other medical reasons, vocational rehabilitation should be utilized to the fullest extent. Plans for vocational rehabilitation preferably should be initiated as soon after admission to the hospital as practical.

(4 *b*) *Post-hospital activity; "open negative"*[3]*:* Unfortunately, in some patients it is not possible to eliminate all cavities with chemotherapy and surgery. Therefore a few patients will be discharged from the hospital in spite of the presence of one or more cavities, providing that the secretions are consistently negative for tubercle bacilli on smear and culture. Excessive fatigue should be avoided by these patients and strenuous labor or recreation curtailed. Isolation is not necessary; suitable employment should be encouraged, but frequent physical examinations, bacteriologic studies, and chest roentgenograms will be required for an indefinite period because of an increased risk of relapse in this group.

(5) *Prophylactic chemotherapy and activity:* Individuals for whom antituberculosis chemotherapy is prescribed for prophylactic reasons, i.e., recent conversion of the tuberculin reaction, the application of prolonged corticosteroid therapy in the presence of a positive tuberculin reaction, et cetera, may continue to work and otherwise lead normal lives.

(6) *Bacteriologically positive with "drug-resistant organisms":* Although with an effective antituberculosis chemotherapy program rest is no longer of major im-

[2] To designate sputum as "negative," it is recommended that three adequate specimens of sputum or of gastric contents be found negative for tubercle bacilli by culture or by animal inoculation. Sputum smears, if done, must also be negative for tubercle bacilli by microscopic examination.

[3] The "open-negative" problem, Amer Rev Resp Dis, 1959, *80*, 118.

portance, it must be recognized that observations in earlier decades almost universally have attested to the value of restriction of activity in controlling or healing the tuberculous process. Therefore the Committee believes that a modified rest regimen still should be prescribed for all patients who harbor organisms which are not susceptible *in vitro* to at least two of the three principal antituberculosis drugs: isoniazid, streptomycin, and para-aminosalicylic acid. Chemotherapeutic regimens which do not include a combination of two of these drugs are not so predictably effective that modified rest treatment can be substantially altered or eliminated.

(7) *Bone and joint tuberculosis:* Restriction of activity must continue to be prescribed on an individual basis for patients with tuberculous problems which obviously would be affected adversely by continuation of normal activity, i.e., tuberculous involvement of weight-bearing bones and joints. Rest designed to eliminate weight-bearing must be enforced until the disease process has been reversed sufficiently to prevent a purely mechanical derangement which might permanently incapacitate the individual.

This Committee still believes that the initial hospitalization of tuberculous patients is necessary even when rest in bed is not indicated. Confinement in a tuberculosis or general hospital during the initial phases of treatment is important for the following reasons:

1. Isolation of the patient is assured while he is still bacteriologically positive or while the bacteriologic status is being established.
2. Superior diagnostic facilities are available for establishing the complete diagnosis.
3. Chemotherapy may be initiated while the patient is under careful observation for possible toxic manifestations.
4. The necessity for surgery may be adequately assessed.
5. Education and reassurance of the patient concerning his disease may be accomplished.
6. The adequacy of social and economic conditions for continued post-hospital therapy can be assessed.

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NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1962—Miami Beach, Florida, May 20–24
1963—Denver, Colorado, May 12–16
1964—New York, New York, May 24–28

1962 ANNUAL MEETING OF THE AMERICAN THORACIC SOCIETY

Invitation

The Medical Sessions Committee *INVITES SUBMISSION OF PAPERS* on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1962 ANNUAL MEETING IN MIAMI BEACH, FLORIDA, MAY 20–23.*

Abstracts

(*1*) Abstracts of papers to be offered for presentation should be submitted before *JANUARY 5, 1962* to the Chairman of the Medical Sessions Committee. *EIGHT COPIES* are required for distribution to committee members, the first copy on bond and the remaining seven on onion skin. Abstracts of papers from investigators outside the United States will be most welcome provided they are in English and are submitted with the understanding that all papers accepted must be presented in English at the annual meeting.

(*2*) Abstracts should be limited to a 300-word summary including *IMPORTANT DATA AND CONCLUSIONS*, and should be typed double-spaced on a 60-space line. Charts and tables should be attached to aid in the selection of papers. Abstracts selected for presentation at the Annual Meeting will be published in THE AMERICAN REVIEW OF RESPIRATORY DISEASES. Manuscripts should be double-checked for accuracy before submitting.

(*3*) Reports of interesting or unusual cases will be considered for inclusion in case conferences.

(*4*) The names, most important academic degree, and title (*list only one*), and complete addresses of the senior author and co-authors should be included in each abstract.

(*5*) All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.

(*6*) Authors will be notified by *FEBRUARY 5, 1962*, if their papers have been accepted for presentation.

(*7*) Papers presented at the Annual Meeting must be *ORIGINAL* contributions *NOT PREVIOUSLY PRESENTED OR PUBLISHED*. Medical papers presented at the meeting may be submitted to THE AMERICAN REVIEW OF RESPIRATORY DISEASES by the author if he so desires.

Suggestions

The Medical Sessions Committee is particularly anxious to build the 1962 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman.

Asher Marks, M.D., *Chairman*
Medical Sessions Committee
American Thoracic Society
1790 Broadway
New York 19, New York

NATIONAL TUBERCULOSIS ASSOCIATION FELLOWSHIPS

As the medical section of the National Tuberculosis Association, the American Thoracic Society (formerly the American Trudeau Society) provides fellowships and traineeships for the graduate education of investigators and teachers in the field of respiratory diseases and tuberculosis. The following types are offered:

Edward Livingston Trudeau Fellowships: A few fellowships at a higher level of training and award are offered to specially qualified candidates with an M.D. degree who have been assured of a continued teaching or research appointment upon completion of training. Trudeau Fellowships may be renewed up to a total period of four years.

Research Fellowships: Postdoctoral fellowships are offered to candidates holding the degree of M.D., Ph.D., or Sc.D. for further training as scientific investigators.

Predoctoral fellowships are offered to graduate students with a bachelor's or master's degree to work on a research project for an advanced degree other than M.D.

Teaching Traineeships: Physicians entering their second or third year of residency in Internal Medicine or Thoracic Surgery at a medical center and interested in a career of medical teaching are offered traineeships toward that end.

Each applicant for a National Tuberculosis Association fellowship or traineeship must have been accepted by the head of the department under whom he expects to work for the next academic year in this country. Awards are determined by individual circumstances and are paid directly to the Fellow on a quarterly basis. They are granted for one year, beginning on any date after April first, and are renewable for a total of three years.

All applications must be submitted by November 1st. Further information about fellowships may be obtained upon request from:

Director of Medical Education
American Thoracic Society
1790 Broadway
New York 19, New York.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

ABSTRACTS

Edited by EDWIN D. KILBOURNE

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The Editor recognizes the inevitable incompleteness and arbitrary selectivity of any abstracting service, including this one. It is his belief that the usefulness of the present Abstracts section will be enhanced if the reader is informed of the general purpose of the abstracts and the criteria employed in their selection.

It is the purpose of the Abstracts section to acquaint the diversified and international readership of the REVIEW with the gist of original and scientific articles which are related to the general subjects of tuberculosis or respiratory diseases implicit in the title of the journal. In this connection, 40 abstracters systematically review 239 journals which represent 36 countries and which are published in 22 languages. In most instances, abstracters cover journals in fields of their special competence. It is evident that the diverse interests of the readers of the REVIEW dictate a balanced selection of clinical, laboratory, and epidemiologic studies. Such selection will often preclude the publication of articles equal in scientific merit to many presented here.

In the publication of abstracts, cognizance is taken of the extent to which the journals yielding them are circulated. Articles from journals of wide circulation may be mentioned by title only. This space-saving device is also employed for articles which defy condensation without the omission of essential data or discussion. The attention of readers is thus directed to the existence of papers which are no less important because they are not summarized.

(Requests for further information on abstracted articles should be directed to the author of the article at the address indicated by an asterisk (*) in each abstract title.)

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NONTUBERCULOUS THORACO-PULMONARY DISEASE

Chronic Pneumonitis with Abscess Formation (in German). G. Mészáros*. *Z Tuberk*, October, 1960, 116: 38–49. (*Tuberkuloseklinik, Debrecen, Hungary)

Pulmonary resection was performed on 26 patients with chronic pneumonitis abscesses, whereas 11 other patients were treated conservatively. Pulmonary resection was thought to be the treatment of choice whenever possible.—J. Haapanen

Pulmonary Allescheriasis. R. E. Travis*, E. W. Ulrich, and S. Phillips. *Ann Intern Med*, January, 1961, 54: 141–152. (*V.A. Hosp., Memphis, Tenn.)

Allescheria boydii is one of the most clearly defined etiologic agents of mycetomas, and it is the most frequent cause of this condition in the United States. Case reports of *A. boydii* infections usually describe some trauma which may have permitted the organism, along with a foreign body, to gain entry into the tissues. This suggests the probability that the fungus is a natural saprophyte, and this has been supported by numerous isolations of the organism from the soil, from a polluted stream, and from several sewage-treatment plants. A case is presented of pulmonary sarcoidosis complicated by an infection of the lungs with *A. boydii*. This complication occurred after repeated courses of antimicrobials and prolonged corticosteroid therapy. The medical history of this fungus is reviewed, and the factors predisposing the patient to the development of this rare infection of the lungs are discussed. This is the second known case report of pulmonary allescheriasis.—T. H. Noehren

The Psychogenic Factor in Asthma in Children (in Portuguese). A. Murce*. *Rev Bras Med*, January, 1961, 18: 567. (*Pediatrician, Providencia Social, Rio de Janeiro, Brazil)

A review is made of typical cases of asthma in children due to psychogenic factors, and two main aspects of the problem are stressed: the patient's susceptibility to "suggestion," and the family and school environments. The first factor involves the temperament of the child, the degree of suggestibility, fear, remembrance of previous crises, et cetera. The family attitude is considered to be a very important factor, as well as day-to-day activities. In conclusion, to achieve results all facets of the problem have to be considered.
—L. de Mello

Incorrect Diagnosis of Cavitary Tuberculosis in Pulmonary Cystic Disease: Clinical and Pathologic Study of Eight Cases (in Italian). N. Belli*, L. Ferri, and S. Rossi. *Lotta Tuberc*, No. 9, 1960, 30: 689–713. (*U. of Rome, Italy)

The clinical history, roentgenographic and bronchoscopic results, and pathologic findings in 8 surgically treated cases of either localized or diffuse pulmonary cystic disease are given in detail. All of the patients had originally been considered cases of pulmonary tuberculosis and were treated accordingly. In most of the cases, the epithelium lining the cavities was of the stratified columnar type; in a few cases it was of the cuboidal type with some aspects of metaplasia. The structure of the submucosa was typically disorganized. An inflammatory reaction was frequently observed in the submucosa; the signs of a tuberculous process were never found. In all of the patients the examination of sputum for the presence of *M. tuberculosis* was repeatedly negative. In 3 patients the roentgenographic pictures clearly revealed the nature of the disease, but in the remaining 5 only the pathologic examination provided a definite diagnosis.—I. Archetti

Spontaneous Chylothorax. J. Schwartz*. *JAMA*, December 17, 1960, 174: 2033–2035. (*Maimonides Hosp., Brooklyn, N. Y.)

A left pleural effusion developed suddenly in a 35-year-old woman. Thoracentesis revealed the presence of creamy fluid which settled in 2 layers on standing, had a specific gravity of 1.012, and cleared when mixed with ether. After removal of 300 ml. of fluid, the patient was treated by bed rest alone. The effusion cleared completely, without need for further aspiration. There was no indication of underlying malignant disease and no history of trauma.—H. Abeles

Paper Electrophoresis in the Differential Diagnosis of Pulmonary Diseases (in German). K.-J. Schröder*. *Z Tuberk*, October, 1960, 116: 132–142. (*Lungenkrankenhaus, Leipzig, Germany)

Electrophoretic examination of serum proteins was performed on 256 patients with pulmonary tuberculosis. In bronchial cancer the albumin was low and the α-globulin high, in tuberculosis the albumin was low and the α-globulin high, in 50 per cent of the cases of benign lymphogranulomatosis the γ-globulin was elevated, and in chronic pneumonia the α_2- and γ-fractions were the most elevated. Electrophoresis did not reveal any specific changes in the different pulmonary diseases.
—J. Haapanen

Eaton Agent Pneumonia. R. M. CHANOCK*, M. A. MUFSON, H. H. BLOOM, W. D. JAMES, H. H. FOX, and J. R. KINGSTON. *JAMA*, January 21, 1961, 175: 213–220. (*National Institutes of Health, Bethesda, Md.)

In a controlled 6-month study at a Marine recruit base, infection with Eaton agent was detected in 68 per cent of 238 men with atypical pneumonia. Infection occurred significantly less often among recruits without respiratory illness. Fluorescent antibody studies suggested that 44 per cent of the recruits were infected at some time during the 3-month training period. Only 1 in 30 infections was manifest as clinically diagnosed pneumonia. The data suggested that the Eaton agent was not highly communicable, an attribute which favored its persistence in the recruits.—H. ABELES

Eaton Agent Pneumonia. J. R. KINGSTON*, R. M. CHANOCK, M. A. MUFSON, L. P. HELLMAN, H. H. FOX, M. A. MANKO, and J. BOYERS. *JAMA*, April 15, 1961, 176: 118–123. (*Navy Bureau of Med., Washington, D. C.)

A controlled double-blind study was carried out to evaluate the efficacy of demethylchlortetracycline in primary atypical pneumonia associated with Eaton agent. By randomization, 290 bacteriologically-negative patients with pneumonia were distributed among a test series of patients who received 0.9 gm. of the drug per day for 6 days and a control series who received a placebo. The drug significantly reduced the duration of fever, rales, cough, malaise, and fatigue. Treatment stopped the progression of the disease and accelerated the clearance of pulmonary infiltration. The findings suggested that the drug had a direct action upon the pulmonary disease process.
—H. ABELES

Electrocardiographic Findings in Patients with Chronic Cor Pulmonale (in Japanese). H. SASAMOTO*, K. HOSONO, K. KATAYAMA, K. TAKAGI, Y. NAKANO, H. TAMURA, and T. DATE. *Resp Circ*, January, 1961, 9: 55–64. (*Keio U. School of Med., Tokyo, Japan)

The relation between the electrocardiographic findings and the pulmonary arterial pressure levels was studied in 153 patients with chronic pulmonary diseases. The presence of tall P waves of more than 0.2 mV in leads II and III or a right axis deviation of more than 90 degrees were usually suggestive of the presence of high pulmonary arterial pressure (mean pulmonary arterial pressure of more than 16 mm. Hg.), although exceptions were found. A few electrocardiographic findings fulfilled the criteria of right ventricular hypertrophy proposed by Sokolov and Lyon (1949), but it was not always possible to associate these findings with the high pulmonary arterial pressure. The mean pulmonary arterial pressure was more than 24 mm. Hg. in all of the patients with an R/S ratio of more than 1.0 in leads V_1 or V_{5R} and ST-T change in V_1 and V_2, although some patients with a mean pulmonary arterial pressure of more than 24 mm. Hg. had no abnormal electrocardiographic findings.—I. TATENO

Clinical and Pathologic Studies in 8 Cases of Chronic Cor Pulmonale (in Japanese). H. SASAMOTO*, R. IGA, Y. NAKAMURA, K. TAKAGI, T. DATE, and H. TAMURA. *Resp Circ*, January, 1961, 9: 65–70. (*Keio U. School of Med., Tokyo, Japan)

Clinical and pathologic studies were performed in 8 patients with chronic cor pulmonale of varied etiology. The signs and symptoms of congestive heart failure were present in only 2 patients during life, but chronic congestion of the liver was found in 7 cases at autopsy. The electrocardiographic findings fulfilling the criteria of right ventricular hypertrophy (Sokolov and Lyon, 1949) were present in most patients in whom this condition was confirmed at autopsy. The presence of peaked P waves was not, however, always associated with right auricular dilatation or hypertrophy. The writers used their own methods of measurement and standards of criteria for the anatomic diagnosis of right ventricular hypertrophy. The thickness of the right ventricle was measured near the pulmonary arterial conus, and that of the left ventricle was measured at a site near the aortic orifice which preferably had minimal development of trabeculae. The total thickness of the heart minus the thicknesses of the endocardium and the pericardium was designated as the true thickness of the myocardium. The normal range for the right and left ventricular walls was less than 4 mm. and 13 mm., respectively. It is of interest that left ventricular hypertrophy was found in 4 cases, peptic ulcer in 3, and marked adrenal atrophy in 1 case.—I. TATENO

Serum LHD Activity in Pulmonary Embolism Diagnosis. W. E. C. WACKER* and P. J. SNODGRASS. *JAMA*, December 24, 1960, 174: 2142–2145. (*Harvard Med. School, Boston, Mass.)

A significant rise in the serum lactic dehydrogenase (LHD) activity occurred in 17 patients with pulmonary embolism or infarction. When employed in conjunction with the measurement of serum bilirubin concentration and the serum glutamic oxaloacetic transaminase activity, the serum LHD activity may provide a specific method for diagnosing pulmonary infarction.—H. ABELES

Medical Mediastinal Emphysema. G. P. Bodey*. *Ann Intern Med,* January, 1961, 54: 46–56. (*Johns Hopkins U., Baltimore, Md.)

Mediastinal emphysema arising either spontaneously or due to some underlying disease is called "medical mediastinal emphysema." This term excludes all cases resulting from trauma or from surgical, diagnostic, or therapeutic procedures. A patient with medical mediastinal emphysema who was studied on the medical wards of the Johns Hopkins Hospital is reported in this paper, along with all 15 cases of this disorder seen at the hospital in a 23-year period. A review of the literature concerning the etiology, symptomatology, pathophysiology, and treatment of this condition is included. Medical mediastinal emphysema is a rare disease. The most common cause in this series is spontaneous mediastinal emphysema. It affects males predominantly, and occurs most frequently in the 11- to 30-year age group. It is almost always a benign disease, but may be potentially serious. The most common signs and symptoms are dyspnea, chest or back pain, subcutaneous emphysema, Hamman's sign, and air in the mediastinum on roentgenographic examination. Air enters the mediastinum from ruptured alveoli and travels along the blood vessel sheaths. Treatment is rarely necessary, but the simplest and most efficacious method is breathing 95 per cent oxygen by mask.—T. H. Noehren

Pulmonary Emphysema: Etiologic Factors and Clinical Forms. D. M. Richards*. *Ann Intern Med,* December, 1960, 53: 1105–1108. (*College of Physicians and Surgeons, New York, N. Y.)

Chronic pulmonary emphysema is considered in terms of the major etiologic forces producing the disease. Three basic forms are described: (*a*) chronic bronchitis and emphysema, (*b*) pulmonary fibrosis and emphysema, and (*c*) nonobstructive bullous emphysema. Within the category of chronic bronchitis and emphysema, two chief clinical forms can be distinguished: (*a*) obstructive emphysema with ventilatory insufficiency, and (*b*) obstructive emphysema with ventilatory insufficiency and alveolar hypoventilation. In the emphysema associated with pulmonary fibrosis, the basic etiologic forces are: (*a*) traction, and (*b*) obstruction. The class of primarily nonobstructive bullous emphysema is presented as a disease in which the etiologic factor is an essential destruction or "atrophy" of respiratory tissues. Secondary air-passage obstruction, however, occurs from various mechanical causes, and is an important and almost inevitable complication in this disease (after author's summary).—T. H. Noehren

Fibroxanthoma of the Lung with Bronchial Involvement. U. Ising* and E. Linder. *Acta Path Microbiol Scand,* Fasc. 2, 1961, 51: 103–122. (*U. of Lund, Sweden)

Three cases are described of fibroxanthoma of the lung, one situated intrabronchially. The other two were connected with a bronchus. Eight hitherto published cases are tabulated. The genesis and nature of fibroxanthoma must still be regarded as obscure.—E. Dunner

Pulmonary Eosinophilic Granuloma: A Clinical and Pathologic Discussion. A. W. Williams*, W. G. Dunnington, and S. J. Berte. *Ann Intern Med,* January, 1961, 54: 30–45. (*V.A. Hosp., Albuquerque, N. M.)

Since 1951, 45 biopsy-proved cases of pulmonary eosinophilic granuloma (histiocytosis X) have been reported. A general concept may be formulated from an analysis of the clinical and pathologic features. This particular manifestation of histiocytosis X is seen most often in white males in the younger age group. The symptoms, if any, are usually mild, and cough is a common complaint. A variety of changes are seen on the chest film, but a diffuse, small, nodular type of infiltration is most common. Small cystic areas are also seen. Biopsies of extrapulmonary sites are almost always unproductive. Lung biopsy is the only certain method of diagnosis. The pathologic picture is the same as that seen in eosinophilic granuloma of bone, and supports the concept that pulmonary involvement is a manifestation of disseminated histiocytosis X. Sites of extrapulmonary change may be found in the bones and central nervous system. However, from clinical observations, patients presenting with pulmonary histiocytosis usually do not show evidence of involvement in other organ systems. Pulmonary fibrosis and cystic changes of the lung may result from pulmonary eosinophilic granuloma, and death from cor pulmonale and respiratory failure may occur. However, as judged from the available follow-up information from 45 cases, the lesions tend to regress, and clinical improvement, rather than progression to pulmonary insufficiency, or at least stability, is most often seen; it is not possible to conclude whether any type of therapy (or none at all) hastens regression. Continued and more nearly complete follow-up data are, of course, needed. Finally, pulmonary eosinophilic granuloma must be considered in the differential diagnosis of the patient with few or no symptoms and a chest roentgenogram showing diffuse nodular infiltrations. As diagnostic lung biopsy is more widely employed, it is anticipated that many more instances of this disease will be encountered. (after authors' summary).—T. H. Noehren

Pulmonary Hamartoma (in Czech). R. Metys*. *Cas Lek Cesk*, February 26, 1960, 99: 277–282. (*Ústav pro doškolování Lékařů, Prague, Czechoslovakia)

Pulmonary hamartoma is a congenital malformation characterized by firm, sharply defined nodules usually located in the periphery of the lung. Histologically, they are mainly cartilages, but they can contain some other tissues as well. Clinically, they are quite without physical manifestations. On roentgenographic examination they are observed in the form of solitary nodules without surrounding reaction. They are usually stationary, but some of them exhibit slow growth. They are considered benign, but can devolop into chondrosarcoma, and surgical removal is justified for this reason. The incidence is not as rare as supposed. During mass roentgenography, hamartomas are found quite often. The author describes 6 such cases. Surgical removal and histologic examination confirmed the diagnosis in 3 cases.—J. Ilavsky

Some Clinical and Metabolic Effects of Topically Applied Hydrocortisone in Exudative Pleurisy (in Slovak). J. Hrnčiar* and J. Gunčaga. *Brat Lek Listy*, February 29, 1960, 40: 242–245. (*Hospital, Banská Bystrica, Czechoslovakia)

Intrapleural application of hydrocortisone in exudative pleurisy in doses from 25 to 50 mg. every 3 to 5 days had no significant effect on the clinical course of the disease. Decrease of the plasma potassium concentration was the most significant metabolic effect observed, followed by decrease of the potassium concentration in the exudate.—J. Ilavsky

The Differential Diagnosis of Pulmonary Round Infiltrations (in German). A. Flecker* and H. Moder. *Z Tuberk*, October, 1960, 116: 24–37. (*Lungenheilstätte Enzenbach (Graz), Austria)

A round lesion was seen in 204 instances in 21,282 patients with pulmonary disease. In 94 cases there were also signs of active pulmonary tuberculosis. In 4 cases there were multiple metastases of cancer with undiagnosed primary tumor. The remaining 106 cases of solitary round lesions were found in 28 patients with cancer, 44 with tuberculosis, 28 with benign lesions, and 6 with metastases. Further analysis revealed that cancer was more frequent in the older age groups. The larger the lesion the higher was the incidence of cancer. Tuberculous lesions were more frequent in the upper lung fields, and the "benign lesions" were found more often in the lower lung fields.—J. Haapanen

Inflammatory Versus Malignant Middle Lobe Disease. L. Virkkula*. *Ann Chir Gynaec Fenn*, Fasc. 1, 1961, 50: 88–96. (*Kirurgi, Helsinki, Finland)

Of 50 cases of middle lobe atelectasis in a surgical series, cancer was found in 27 cases and the remainder were inflammatory. Only 1 case of cancer was revealed in 9 female patients, in contrast to 26 in 41 men. The diagnosis of cancer was made by cytologic examination in 1 case and by bronchoscopy in 21 cases.—J. Haapanen

Excavating Pulmonary Metastases. G. D. Dodd* and J. J. Boyle. *Amer J Roentgenol*, February, 1961, 85: 277–293. (*M. D. Anderson Hosp., Houston, Texas)

In a series of 6,729 consecutive patients seen in a tumor institution, excavation was encountered in 9 per cent of patients with primary tumors of the lung and 4 per cent of patients with pulmonary metastases. Although relatively nonspecific, the identification of excavating pulmonary metastases does permit some generalization concerning their origin. Approximately 70 per cent will be of squamous cell origin. In the male, the bulk arises from tumors of the head and neck, and in the female, from the genitalia. The number of adenocarcinomas has been small, but there was no significant sex preference in the cases seen, and the pulmonary forms were indistinguishable from the squamous variety. In general, the metastases originating in the head and neck undergo cavitation when quite small, and have extremely thin walls. The excavating lesions of the bladder and genitalia have, on the whole, thicker walls and resemble the more common form of excavating pulmonary neoplasm. The possible mechanisms of excavation are discussed. It is concluded that specific tissue characteristics are primarily responsible for the majority of roentgenographically detectable excavating metastases rather than deficiency in the blood supply per se.—T. H. Noehren

Positive Middlebrook-Dubos Reactions in Nonspecific Pulmonary Diseases (in Slovak). E. Schwartz* and J. Čunderlík. *Rozhl Tuberk*, June, 1959, 19: 429–438. (*Tuberculosis Hosp., Podunajské Biskupice, Czechoslovakia)

Three modifications of the Middlebrook-Dubos hemagglutination and hemolysin tests were used in this study of 207 patients with various chronic nontuberculous diseases. Thirty patients had positive reactions with the test method as described by Dubos and Middlebrook. About the same results were obtained if the tested sera had been treated with tuberculin prior to the tests. When the tested sera were pretreated with bacterial suspensions prepared from nonspecific bacterial populations isolated from the patients concerned instead of with Old Tuberculin, only 4 of the 30 sera again

gave positive results. These findings are considered to be proof that nonspecific bacteria such as Staphylococcus and Streptococcus cause the formation of antibodies which can give positive Middlebrook-Dubos reactions in dilutions of 1:16 and higher.
—J. Ilavsky

Diffuse Lung Metastases as the Only Manifestations of a Primary Sarcoma of the Right Auricle of the Heart (in French). R. Depierre*, G. Chomette, R. Abelanet, J. Pointillart, and J. P. Gubler. *Presse Med*, January 25, 1961, 69: 159–160. (*Hosp. Paul-Brousse, Villejuif, Seine, France)

A young woman was admitted to hospital with the symptoms of dyspnea, chest pain, and fever. A roentgenogram of the chest revealed diffuse nodular infiltrations and a small pleural effusion at the right base. The fluid removed for diagnostic purpose was hemorrhagic. The Mantoux test was positive and the sedimentation rate elevated. The patient was treated with antituberculosis drugs, but the pulmonary lesions increased in size and a small pneumonthorax appeared. The temperature, however, returned to normal. Finally, a pulmonary biopsy was performed which established the diagnosis of metastases of a fibroblastic sarcoma. The patient died after six months of illness. The autopsy revealed the primary tumor to be located in the right auricle of the heart. The tumor was defined as rhabdomyoma. The metastases found in the biopsy material were not sufficiently differentiated histologically so as to provide a clue to the nature and location of the primary tumor.
—E. Lyon

Acquired Lung Syphilis (in German). W. Schulte-Brinkmann* and R. M. Konrad. *Z Tuberk*, October, 1960, 116: 143–148. (*Chirurgische Klinik, Düsseldorf, Germany)

Pneumonectomy was performed on a 47-year-old female for lung syphilis with secondary changes. Despite antiluetic treatment the seroreactions remained positive for 2½ years.
—J. Haapanen

***Trichosporon Capitatum* in a Patient with Lung Cavity** (in Czech). L. Melichar*, J. Sobotkiewicz, and P. Frágner. *Rozhl Tuberk*, August, 1960, 20: 552–555. (*Hospital, Mladá Boleslav, Czechoslovakia)

Trichosporon capitatum Diddens-Lodder was repeatedly cultivated from the sputum of a patient with a nonspecific lung cavity. After nystatin and cabimicina (Trichomycin®) treatment, there was both subjective and objective improvement. The cavity, originally skiascopically visible, was no longer demonstrable. Despite this, Trichosporon was again cultivated from this patient's sputum.
—J. Ilavsky

TUBERCULOSIS, PULMONARY

Tuberculosis of the Aged (in Czech). M. Kratochvil*. *Rozhl Tuberk*, April, 1960, 20: 309–318. (*Hospital, Ústí nad Orlici, Czechoslovakia)

The incidence of pulmonary tuberculosis in persons up to and older than 60 years was studied and compared. No substantial differences were found.
—J. Ilavsky

Contribution to the Study of Gallbladder Disease in Pulmonary Tuberculosis (in Italian). A. Carrata Thomes*. *Gior Ital Tuberc*, No. 3, 1960, 14: 150–163. (*Sanatorio S. Luigi Gonzaga, Torino, Italy)

The relationship between pulmonary tuberculosis and gallbladder disease was studied in 46 patients of both sexes with various forms of tuberculosis. Patients were selected in part during a 4-year period when the use of antimicrobials had just started, and others were chosen during a period when antimicrobials were already the basis of treatment of tuberculosis. The frequency of gallbladder disease was low (from 0 to 1.8 per cent), and it was 8 times higher in the second group than in the first. It occurred especially among patients of middle age, and a lower prevalence in men than in women was observed. It was noted that gallbladder disease was more often present when tuberculosis was either chronic or very severe. The 46 cases were distinguished, following cholecystography, in 7 cases with and in 39 cases without gallstones. The symptomatology and objective signs are described and the diagnosis is discussed. The conclusions of the study, based upon the present observations and data from the literature, were as follows: tuberculous cholecystopathy rarely exists without cholecystolithiasis, and it was not observed in the present patients. Cholecystopathies were rare in general, particularly in association with gallstones; the rate of incidence of the stone-free form was 1:175. Finally, it can be said that the various methods of tuberculous therapy, both medical and surgical, can favor gallbladder disease.—I. Archetti

Contribution to the Study of the Histopathogenesis of Pulmonary Tuberculous Lesions. III. Cavities (in Italian). Q. Lunetta*. *Ann Med Sondalo*, No. 5, 1960, 8: 296–314. (*Villaggio Sanatoriale, Sondalo, Italy)

Forty cases of tuberculous cavities in different stages of evolution which were treated with anti-

microbials and then by exeresis were investigated by histologic methods in order to study their pathogenesis. The cavities were divided into 2 groups: the truly empty cavities, and those filled with caseous material (only 2 cases), which represent a stage of evolution of the tuberculous lesion and are in a phase of inactivity. The distinction was based on histologic as well as clinical and roentgenographic data. The different phases of evolution of the empty cavities are given, and the various stages through which the lesion goes to reach the inactive phase are described. Two different systems of connective tissue were recognized in the lesions: the main pericavitary system; and the secondary, due to histologic reactions around the various granulomatous foci. The presence of a capillary net in the latter is emphasized as an important determinant in the further evolution of the cavity itself. The use of the Hotchkiss staining method for the study of cavities is stressed, since it might give data concerning the orientation of the local histogenic reaction which normal morphologic research does not afford.
—I. Archetti

Diffuse Caseous Lymphadenitis of the Mediastinal and Mesenteric Lymph Nodes in an Adult (in Italian). N. Belli* and E. Cutillo. *Ann Ist Forlanini*, No. 4, 1959, 19: 509–520. (*U. of Rome, Italy)

Diffuse caseous lymphadenitis is a rare finding in populations in which tuberculosis is endemic, and it has been observed in white persons only in cases of serious physical and alimentary ailments. A case in a 32-year-old man who died suddenly with congestion and cerebral edema is described both clinically and pathologically. At autopsy, a systemic and diffuse caseation of the mediastinal and mesenteric lymph nodes in the course of primary tuberculosis was found. The pathogenesis of this rare localization was attributed to an exaggerated individual susceptibility and to the use of antimicrobials. The drugs probably prevented diffusion of the process and localized it in the lymph nodes.—I. Archetti

The Fate of Negative Cavities in Patients Discharged from Institutional Treatment (in Slovak). L. Minárik*, S. Mečíř, and L. Ürge. *Rozhl Tuberk*, August, 1960, 20: 509–518. (*Sanatorium, Vyšné Hágy, Czechoslovakia)

One hundred and one patients with negative cavities (unassociated with culturable tubercle bacilli) were followed up from 1 to 5 years after they were discharged from institutional treatment. In 32 cases there was reactivation of tuberculosis, and in 42 cases the cavities closed completely. The rest remained unchanged.—J. Ilavsky

The Pathogenesis of Pulmonary Tuberculoma (in Slovak). T. Skalský*. *Rozhl Tuberk*, July, 1959, 19: 547–548. (*Sanatorium, Tatranská Polianka, Czechoslovakia)

The development of a pulmonary tuberculoma is rarely observed and followed up. The author describes a case in which pulmonary tuberculoma developed during the hospitalization period and which was studied clinically and roentgenographically. It started as tuberculous bronchitis, with a light peribronchial infiltration. Very soon, an oval infiltrate developed and its size increased considerably during the 2 months of roentgenographic follow-up study. Histologic examination of the surgically removed sample confirmed the diagnosis.
—J. Ilavsky

New Therapeutic Regimen in Tuberculosis Hospitals (in Czech). O. Dymer*, J. Kvapil, and F. Polánsky. *Rozhl Tuberk*, March, 1960, 20: 194–200. (*Krajská nemocnice KNV, Prague, Czechoslovakia)

The usual institutional therapeutic regimen was modified and gradual activity was substituted for bed rest. Physical exercise, work, and organized cultural activities were started from the beginning of institutional treatment. Patients were divided into 3 groups according to their state of clinical compensation. Bed rest was supplemented with passive and active exercises even in decompensated cases. Subcompensated cases usually rested 3½ hours a day, did 10 minutes of physical training, took part in sports or games for 30 minutes, and worked for 30 minutes. The patients of the second group gradually increased the time for sports and work until they reached the third grade, in which they had 2 30-minute periods for sports and from 2 to 4 30-minute periods for work. Maintenance, gardening, and the assembling of spare parts for calculating machines are usually performed by patients. This new regime is very beneficial psychologically and keeps patients in good physical condition. Not even temporary unfavorable effects were observed.—J. Ilavsky

Pulmonary Tuberculosis Due to Resistant Bacilli in Patients without Prechemotherapy (in French). R. Thibier*, G. Canetti, J. Gosset, and J. N. Vivien. *Rev Tuberc (Par)*, July–August, 1960, 24: 831–865. (*Centre E. Rist, Paris, France)

The incidence of drug-resistant tubercle bacilli was studied in patients without previous chemotherapy in a Parisian institution for students. Most

of the patients were between the ages of 15 and 25; in 50 per cent, tuberculosis had been discovered by routine roentgenographic examination. Susceptibility studies were performed prior to the institution of chemotherapy. Cultures were grown on Löwenstein-Jensen medium for 8 weeks. The criteria for bacterial resistance were as follows: isoniazid resistance—100 per cent of bacilli resistant to 0.1 γ of isoniazid per ml. or 1 per cent resistant to 0.2 γ of isoniazid per ml.; streptomycin resistance—all strains containing more than 1 per cent of bacilli resistant to 4 γ of streptomycin per ml.; PAS resistance—all strains containing more than 1 per cent of bacilli resistant to 0.5 γ of PAS per ml. Of 226 newly-admitted patients who had never received antituberculous chemotherapy, 61 per cent had cultures positive for tubercle bacilli on admission; 86 per cent of these patients were susceptible to all 3 antituberculous drugs, and 14 per cent were resistant to 1 or more drugs. In order to determine the incidence of bacilli initially resistant to a given drug, the authors calculated the incidence in relation to all patients with sputum positive for tubercle bacilli who had never received this particular drug, even if they had been previously treated with another drug. After this correction, the incidence of initial isoniazid resistance was 9 per cent; that of streptomycin resistance, 9 per cent; and that of PAS resistance, 0.7 per cent. There was resistance to 2 drugs in 5 per cent of the nontreated patients. Thus, the incidence of initial drug resistance was by no means low. The degree of resistance was generally high. Clinically, the course of tuberculosis due to initially-resistant bacilli could not be distinguished from that due to susceptible strains. Among this small number of cases, there was no indication that the virulence of resistant bacilli was attenuated for man as it is for guinea pigs. The treatment results in the 21 cases were: excellent, 11 cases; favorable, 5 cases; and unfavorable, 5 cases. In 10 patients who were susceptible to 2 drugs, there were 8 excellent results, as compared with 3 excellent results in 11 patients who were susceptible to 1 drug. There were 5 unfavorable results in patients susceptible to only 1 drug, while there were no unfavorable results among patients susceptible to 2 drugs. Since the initial treatment in both groups included the drug to which the patients' bacilli were found to be resistant, these figures further confirm the authors' view that the maintenance of a drug to which there is *in vitro* resistance is useless. The incidence of initially-resistant strains is sufficiently high, at least in Paris, to warrant triple therapy in new cases, pending the result of susceptibility studies.
—V. Leites

Postepy Hig Med Dosw, No. 2, 1960: Resection of Lung Tissue in Tuberculosis: Proceedings of the Phthisio-surgical Conference in Zakopane, November 21–22, 1958:

The Histobacteriology of Tuberculous Lesions in Resected Specimens. E. Waniewski. P. 112:

Non-necrotic lesions (such as tuberculous bronchopneumonia and perifocal reactions) are practically never encountered in resected pulmonary tissue as they are resorbed under the influence of chemotherapy. Necrotic lesions (tuberculomas) contain bacilli in focal distribution, which may be observed in histologic sections. Bacilli from these lesions are not viable, i.e., they cannot be cultivated and do not infect laboratory animals. It is not certain whether these bacilli are dead, but it is believed that chemotherapeutic agents and some metabolites of tissue necrosis are strongly tuberculostatic, or even tuberculocidal. Chemotherapy is thought to lead to nonviability of the bacilli in closed lesions, although this is not supported by the results of prechemotherapy studies in which nonviable bacilli were also encountered in closed lesions. Tuberculous cavities in specimens resected after chemotherapy contain focally distributed, viable, and culturable tubercle bacilli, sometimes in large numbers. It appears that not chemotherapy, but the morphology of the necrotic lesion, is the decisive factor causing the death of the bacilli (if they are really dead). Bacilli have rarely been encountered outside necrotic areas. Tubercle bacilli are very rarely found in histologic sections of fibrotic or cicatricial parenchymatous lesions, or in tubercle and giant cells. Studies of the behavior of the tubercle bacillus in the host organism have contributed to the clarification of many problems connected with the modern therapy of tuberculosis (after English summary).

Results of Bacteriologic and Histopathologic Examination of Nodes Removed in Lung Resection. E. Paryski, M. Harazda, and H. Jedrzejewska. P. 118:

The examination of 101 tuberculous foci removed in lung resections in 50 patients included 87 encapsulated nodes and 14 solid tuberculomas. The main pulmonary lesions were not studied. Ten per cent of the examined nodes were excised from the unresected pulmonary parenchyma. Tubercle bacilli had been demonstrated in the sputum of 35 of the 50 patients presurgically. All of the patients received antituberculous drugs such as streptomycin, isoniazid, and PAS, but the chemotherapy was often interrupted and not combined. Eight patients were treated for 6 months, 25 for 6 to 12 months, and 17 for more than a year. The studied nodes were classified into 6 groups according to the content of the caseous material. Histologic examination demonstrated caseation, tuberculous granulation, and numerous vessels in the capsulae of all of the nodes. Lymphocytic in-

filtrations and tuberculous granulation were also present outside the fibrous capsulae, as well as connections with the bronchioles. Microscopy revealed tubercle bacilli to be present in 90 per cent of the nodes; 57 per cent of the bacilli were culturable. A relationship was observed between the percentage of bacilli cultured on medium and the morphologic type of the node. Bacilli were cultured from inflammatory nodes and tuberculomas in 80 per cent of the cases, from closed lesions with soft caseous masses in 60 per cent, from dry caseous lesions in 46 per cent, and from fibrosing and calcifying nodes in 17 per cent. The percentage of culturable tubercle bacilli was higher than that obtained from similar material by other investigators. Since the present material demonstrates that the nodes were active and contained viable bacilli despite previous chemotherapy, it should be taken into consideration that similar lesions remaining in the parenchyma after surgery may be the cause of recurrence of the active process (after English summary).—G. FEDDERS

Extrahilar Eggshell Calcifications in a Case of Silicotuberculosis (in Italian). F. BELLINI* and E. GHISLANDI. *Med. Lavoro,* No. 10, 1960, 51: 600–606. (*U. of Milan, Italy)

Eggshell calcifications of the thoracic lymph nodes, which were seen in 4.7 per cent of 1,905 hospitalized patients observed from 1945 to 1960, are considered almost exclusive to silica pneumoconiosis. The nodes involved were, almost without exception, those of the pulmonary hilum. The author describes a case of silicotuberculosis in a 55-year-old man, with the exceptional finding of numerous eggshell calcifications not only of the visceral lymph nodes but in the thoracic wall as well, i.e., the intercostal spaces and the anterior and posterior mediastinum.—I. ARCHETTI

Unclassified (Atypical) Photochromogenic Mycobacteria as Causes of Chronic Pulmonary Diseases (in Czech). M. KUBÍN*, P. OSTRÝ, J. KRUML, M. SUCHANOVÁ, J. PROŠEK, F. PŘÍBOROVSKÝ, and J. SALZMAN. *Rozhl Tuberk,* May, 1959, 19: 337–349. (*Výzkumný ústav tuberkulósy, Prague, Czechoslovakia)

Five patients in whom unclassified photochromogenic mycobacteria were repeatedly cultivated were thoroughly studied. All had chronic pulmonary processes with cavities. From the clinical point of view, 4 patients did not respond too well to the usual bed rest and chemotherapy; only 1 patient recovered under this therapeutic regimen. Resection was performed in 3 cases with good results. Histologic examination of the removed portions of lung revealed a chronic caseonodular and cavernous process accompanied by proliferative and atrophic bronchitis and marked fibroanthracosis surrounding all lesions. All of the strains of photochromogenic mycobacteria were mutually related, and had decreased virulence for experimental animals.—J. ILAVSKY

Pulmonary Disease in Adults Associated with Unclassified Mycobacteria. L. C. CHRISTIANSON* and H. J. DEWLETT. *Amer J Med,* December, 1960, 29: 980–991. (*U. of Texas Southwestern Med. School, Dallas)

The records of all cases of pulmonary disease associated with the isolation of unclassified mycobacteria observed in the adult tuberculosis division of Parkland Memorial Hospital in 3 years are reviewed in detail. The number of cases represented about 1 per cent of all admissions to the tuberculosis division during the time of the study. The clinical, epidemiologic, therapeutic, and pathologic data in 25 patients with pulmonary lesions from whom the only known pathogenic organism isolated was an unclassified Mycobacterium suggest that unclassified mycobacterial infection of the lung is a definite clinical entity. In this locality the strains that appeared to have clinical significance were photochromogens and nonchromogens. The clinical and pathologic picture is similar in many respects to tuberculosis, but displays a very real difference in the degree of infectiousness and response to treatment. Therapy is not satisfactory with the drugs available at this time, but because of some evidence that pulmonary unclassified mycobacterial disease can be progressive in certain subjects, active infections should be treated. In addition, this study includes 14 patients with apparently active pulmonary disease from whom both *M. tuberculosis* and chromogenic mycobacteria were isolated at various times during their illness. Both of the organisms isolated from this group showed a high level of *in vitro* drug resistance. The clinical course of these patients was indistinguishable from that of far advanced pulmonary tuberculosis, and it was not apparent from this data what role, if any, the unclassified mycobacteria played in their disease.—T. H. NOEHREN

THORACIC SURGERY

Uncommon Location and Development of Bronchial Adenoma (in German). H.-D. CLAUS. *Z Tuberk,* October, 1960, 116: 149–153.

A subsegmentally located bronchial adenoma caused a chronic, almost asymptomatic, abscess. It was first diagnosed as tuberculoma, and after the formation of a check-valve mechanism the diagnosis of hydatid cyst was made. The correct diagnosis was obtained only by thoracotomy.
—J. HAAPANEN

Nitrogen Mustard and the Steroid Hormones in the Treatment of Inoperable Bronchogenic Carcinoma. J. Wolf*, P. Spear, R. Yesner, and M. E. Patno. *Amer J Med*, December, 1960, 29: 1008–1016. (*VA Hosp., Bronx, N. Y.)

Four hundred and ninety-six patients with bronchogenic carcinoma were divided into comparable groups and treated for 3 months with nitrogen mustard, diethylstilbestrol, testosterone propionate, cortisone, progesterone, or an inert compound (lactose). The median survival time for the control group was 93 days, as compared with 121 days for the group receiving nitrogen mustard and 56 days for the patients given cortisone. The estimated mean survival time also confirmed the very slight advantage of nitrogen mustard as compared with the inert compound, as well as demonstrating the accelerated death rate among the patients treated with cortisone. The results with the other drugs were intermediate between those with the inert compound and cortisone, the experience in the group receiving progesterone being almost as bad as that in the group given cortisone.
—T. H. Noehren

Pulmonary Hamartoma (in German). E. Hasche* and V. Haenselt. *Z Tuberk*, October, 1960, 116: 1–23. (*Heilstätten Bad Berka, E. Germany)

A detailed description of 3 intrabronchial and 7 intrapulmonary hamartomas showed that the more frequent intrapulmonary type had no relation to the bronchial system and was subpleural; except for occasional pleural pain, they were clinically asymptomatic and were diagnosed accidentally. In contrast, the uncommon central intrabronchial hamartomas caused characteristic objective and subjective symptoms which, however, were misinterpreted for a period of time. The roentgenographic diagnosis of intrapulmonary hamartoma is based on the structure and localization of the tumor, as well as on the follow-up series of roentgenograms. The intrabronchial variant could be diagnosed only by bronchologic examinations. Intrapulmonary hamartoma is treated by enucleation. If the intrabronchial tumor is followed by bronchial obstruction and atelectasis, it has to be treated surgically, either by extirpation during bronchoscopy or by resection of the bronchus and the corresponding portion of the lung. Histologically, the intrapulmonary hamartomas were adenochondromas, whereas the intrabronchial tumors were lipo- or oesteochondromas.
—J. Haapanen

Endocavitary Drainage Associated with Thoracoplasty (Report on 68 cases) (in Italian). B. Occhialini* and B. Burgio. *Ann Med Sondalo*, No. 1, 1960, 8: 3-22. (*Villaggio Sanatoriale, Sondalo, Italy)

According to Monaldi, there are two types of thoracoplasty associated with endocavitary drainage. First, the so-called thoracoplasty of protection, which is intended to make definitive the results obtained by endocavitary drainage; and the thoracoplasty of integration, employed when endocavitary drainage alone is insufficient to cure the tuberculous process. Of 356 patients treated with the Monaldi method, 68 with cavities of the upper pulmonary lobes were chosen. They were 13 to 66 years of age, and the majority of them were no longer susceptible to antimicrobials. Twelve were treated with the protective thoracoplasty and 56 with integrated thoracoplasty. The results obtained in 55 of these patients were as follows: 49 (89.2 per cent) recovered; 2 (3.6 per cent) improved, and in 4 (7.2 per cent) the treatment was unsuccessful. The history of 8 of these patients is given in some detail. Thoracoplasty should not be started too early, in order that it may be limited in extent and performed when the general condition of the patient is better and more favorable to surgical treatment.—I. Archetti

Non-Collapsing Air-Filled Esophagus in Diseased and Postoperative Chests. G. Blomquist* and P. Mahoney. *Acta Radiol (Stockh)*, No. 1, 1961, 55: 32–42. (*University Hospital of Lund, Sweden)

If the wall of the esophagus becomes fixed by scarring and adhesions to adjacent structures, the lumen may remain patent and fail to collapse. It may then contain air and may be demonstrable on roentgenographic examination of the chest. The condition has been reported in cases of mediastinitis as well as in postoperative chest cases. Noninflammatory scarring and adhesions of the esophageal wall following surgery of the lung and mediastinal tissue is the entity reported. Ten cases are discussed in detail. The typical appearance of the esophagus following operative procedure in the thorax is reported as a vertical, linear, air-containing structure about 1 cm. in diameter. It is often curved, kinked, and deviated to 1 side of the chest. Because of better anchorage in its upper third, the deviation is less apt to be marked in this portion. In the lower chest, however, esophageal deviation is apt to be marked. In addition to surgical procedures, scleroderma, carcinoma, and achalasia may cause rigid esophageal walls, the lumina of which contain air. The differential diagnosis of the air-filled esophagus is considered to be loculated effusions, abscess cavities, lung herniations, and cysts.—E. Shabart

Thoracic Surgery: Medical Progress. J. G. Scannel* and E. W. Wilkins, Jr. *New Engl J Med*, December 22, 1960, 263: 1291–1300. (*Harvard Med. School, Boston, Mass.)

The development of satisfactory extracorporeal circulation has led quite naturally to increased surgical emphasis on the heart and great vessels, and the sensational progress made in the cardiovascular field has obscured the lung and the esophagus. The purpose of the present report was to show that these fields have not lain fallow. Since this is a comprehensive review with 126 references to the literature, only some of the more interesting topics covered are mentioned: unilateral pulmonary-artery occlusion during the course of cardiac catheterization used as a test of lung function; the importance of respiratory acidosis and hypercapnia in the development of postoperative circulatory collapse; staphylococcal and fungal infections; tuberculous and unclassified mycobacterial pulmonary disease; the solitary pulmonary nodule; blood-vessel invasion in cancer of the lung; changes in the bronchial epithelium, with an impressive incidence of carcinoma *in situ* or frank invasive carcinoma elsewhere in the lungs of patients undergoing resection of an obvious bronchogenic carcinoma; and tracheal fenestration in the long-term management of advanced emphysema.—M. J. Small

Relapses after Lung Resection for Pulmonary Tuberculosis (in Italian). P. Zorzoli* and A. Orlandoni. *G Ital Tubere*, No. 3, 1960, 14: 132–140. (*U. of Pavia, Italy)

The incidence of relapses (both reactivation of old processes and reinfections) after various types of lung exeresis was studied in 201 patients with pulmonary tuberculosis. One hundred and seventy-eight (88.5 per cent) were considered recovered: of these, 167 were in the 36th postoperative month. Twenty-three (11.5 per cent) had relapses: of these, 9 recovered, 10 remained under treatment, and 4 died. The analysis of these few cases demonstrates the importance of the pathogenesis of relapse in the early form of the disease (the fibroulcerous forms had most of the relapses, while the cavitary forms had a notably minor incidence of complications), as well as the state of the residual parenchyma and the type of operation performed. Relapses were more frequent when the tuberculous process was less quiescent, and they were 3 times more numerous after upper left lobectomy, in spite of the fact that right lobectomies were performed more often. The rate of relapse was higher during the first 2 years after operation, and then become rare. In order to avoid reinfection, the importance is emphasized of correct indication for resection, adequate antimicrobial treatment after surgery and, particularly, a long period of rest in a sanatorium, preferably in the mountains.—I. Archetti

ROENTGENOGRAPHY

Roentgenologic Aspects of Asbestosis. M. Hurwitz*. *Amer J Roentgenol*, February, 1961, 85: 256–262. (*Johannesburg, South Africa)

The roentgenographic features of asbestosis as seen among asbestos workers in South Africa are described and discussed. Emphasis is placed on the pleural changes, which are found more frequently than is classic diffuse pulmonary fibrosis. Pleural involvement also occurs frequently in association with lung fibrosis. A characteristic pattern of sclerotic pleurisy presenting in the form of calcific pleural plaques is described. Although this pattern may be seen in a number of pneumoconioses resulting from exposure to certain silicate dusts, its incidence in asbestosis has received scant attention in previous publications. Diffuse interstitial fibrosis occurs commonly in combination with the pleural changes described. The diffuse type of fibrosis found in asbestosis should be differentiated etiologically from pulmonary collagenosis. Less frequently, there are lung changes alone, presenting the classic pattern of simple uncomplicated diffuse pulmonary fibrosis. This may vary in degree. There may be merely a homogenous clouding and haze over the lung fields, particularly over the lower zones, producing a ground glass appearance. Some cases present with localized areas of homogenous, compact, pneumonic consolidation and fibrosis. These areas may occur in any part of the lung. They do not appear to be disposed in a regular lobar or segmental pattern as in the pneumonias, nor are there any accompanying infiltrative changes such as may be observed in pulmonary tuberculosis. A well-marked nodular lung pattern presumably due to associated silica exposure is occasionally encountered in asbestos workers. The complications of asbestosis are briefly referred to, including pleural mesothelioma, which appears to be relatively common among asbestos workers in the northern Cape Province of South Africa.—T. H. Noehren

Idiopathic Unilateral Hyperlucent Lung. H. Francis*. *Amer J Roentgenol*, February, 1961, 85: 253–255. (*Taguria, Libya, North Africa)

An additional case is reported demonstrating classic roentgenographic, fluoroscopic, and bronchopulmonary changes of the idiopathic unilateral hyperlucent lung syndrome complicated by acute pneumonia on the abnormal side. This syndrome

includes: (*1*) increased radiolucency of an entire lung or lobe, with a constant normal volume during inspiration and expiration; (*2*) a small hilar shadow with diminution in pulmonary vascularity proved by pulmonary angiography; and (*3*) the peculiar terminal bronchiectatic pattern with lack of alveolar filling but with normal patency and distribution of the major and minor bronchi. Many of the patients previously reported to have this syndrome have had varying degrees of respiratory difficulty. The present case history is not one of recurrent pulmonary infections or of pulmonary symptomatology; in fact, only one severe respiratory illness had occurred prior to the time of the patient's original study. The etiology is not definitely known but, if the vascular abnormality is congenital, the possibility that the bronchiectasis may be acquired is suggested in the present case by the rather severe bout of pneumonia in childhood which may have resulted in permanent changes on the hypovascularized side.
—T. H. NOEHREN

Pulmonary Alveolar Proteinosis. S. H. ROSEN*. *U S Armed Forces Med J*, No. 12, 1960, 11: 1507–1515. (*V. A.—Armed Forces Institute of Pathology, Washington, D. C.)

The scientific exhibits on pulmonary alveolar proteinosis presented at the 108th Annual Meeting of the American Medical Association in 1959, and the 26th Annual Meeting of the American College of Chest Physicians in 1960, have been adapted for publication in this report. A brief discussion of the disease is presented under the following headings: clinical manifestations, diagnosis, course, therapy, prognosis, etiology, laboratory findings, pathology, roentgenography, pulmonary function studies, and the influence of age, sex, race, occupation, and geographic distribution. A series of photographs illustrates the salient findings from roentgenography and from a study of both gross and microscopic clinical specimens from patients with pulmonary alveolar proteinosis. The differences, as well as the similarity, between this disease entity and that of interstitial plasma-cell pneumonitis (pneumocystic pneumonitis) can be observed in a companion series of photographs.—C. H. PIERCE

CHEMOTHERAPY

Therapy with Amphotericin B in North American Blastomycosis. R. S. ABERNATHY* and G. T. JANSEN. *Ann Intern Med*, December, 1960, 53: 1196–1203. (*U. of Arkansas Med. Center, Little Rock)

Seventeen patients with North American blastomycosis have been treated with parenterally administered amphotericin B. Of the 16 who received adequate therapy, 1 patient died during treatment, and another showed no change. Fourteen patients experienced prompt healing with therapy, but 2 later had recurrences of their disease, and another died of other causes shortly after therapy had been terminated. The remaining 11 patients have been followed for an average of 13 months, without recurrence. Toxic reactions to amphotericin B were frequent, and therapy had to be discontinued in 2 patients because of renal toxicity; however, no permanent renal damage has been recognized (after authors' summary).—T. H. NOEHREN

Experiences with Iridozin® (Ethionamide) (in German). P. HOFMANN*, L. NICKEL, and G. JUNGHANS. *Tuberkulosearzt*, January, 1961, 15: 18–26. (*Bakteriol. Anstalt, Regensburg, Germany)

Ethionamide (Iridozin®) proved to be a highly potent tuberculostatic fully effective against isoniazid-resistant strains. Doses of 15 to 20 mg. per kg. of body weight were followed by gastrointestinal disturbances which were severe enough to indicate discontinuation of treatment in 10 to 15 per cent of the cases. The side effects subsided completely after discontinuation of treatment. Fifty per cent of the patients (61 of 123) had a prolonged course of treatment without signs of intolerance.—J. HAAPANEN

Clinical Trial with Iridozin® (in German). R. BICKHARDT* and H. TUCZEK. *Tuberkulosearzt*, January, 1961, 15: 27–32. (*Tbk.-Krankenhaus Gauting/München, Germany)

Ethionamide (Iridozin®) was given to 22 patients with cavitary pulmonary tuberculosis in a dosage of 0.75 to 1.0 gm. daily. Only 9 of these patients were able to finish the planned trial period of 3 months. In the remaining 13 cases the drug had to be discontinued due to gastric, hepatic, and central nervous system disturbances consisting of loss of appetite, reduction of weight, nausea, vomiting, vertigo, depression, restlessness, irritability, and insomnia. Liver function tests and the electrophoretic pattern pointed to impairment of liver function. The addition of promethazine to the regimen did not significantly prevent or reduce the severity of the side effects in 5 patients.
—J. HAAPANEN

A Study of the Glutamic Acid Activity upon Neurotoxic Symptoms Accompanying Isoniazid Therapy (in Czech). V. KÜRTI* and O. NOVÁK. *Rozhl Tuberk*, May, 1959, 19: 389–396. (*Výzkumny ústav tuberkulosy, Prague, Czechoslovakia)

Forty-two patients with evident neurotoxic

symptoms were able to continue isoniazid therapy with doses of up to 10 mg. per kg. of body weight for 6 to 12 months if the isoniazid was administered simultaneously with glutamic acid. In most cases, the ratio of 1 part isoniazid to 4 parts glutamic acid was quite satisfactory. Tolerance of this combination was excellent.—J. Ilavsky

Influence of Isoniazid upon Certain Acids Related to Carbohydrate Metabolism (in Czech). R. Krulik*, M. Kohout, and V. Kürti. *Rozhl Tuberk*, June, 1959, 19: 450–455. (*Tuberculosis Research Institute, Prague, Czechoslovakia)

The effect of isoniazid upon concentrations of pyruvic, citric, and lactic acid in the blood and urine was tested in 10 patients. Three hours following a 300-mg. dose of isoniazid, the pyruvic acid blood concentrations remained unchanged, citric acid was higher in all patients, and lactic acid was higher in 5 patients and unchanged in the other 5. After 24 hours there was a higher urine excretion of pyruvic acid in all patients. The citric and lactic acid contents of urine were higher in 8 patients. The authors were able to demonstrate that pyruvic acid combines with isoniazid, forming pyruvic acid isonicotinoyl hydrazone. The bacteriostatic properties of this hydrazone are equal to those of isoniazid.—J. Ilavsky

The Problem of Rapid Inactivation of Isoniazid (in German). R. Pfaffenberg and H. Jähler. *Z Tuberk*, October, 1960, 116: 121–131.

The rate of inactivation of isoniazid is a characteristic feature of every individual. It is hereditary and independent of tuberculosis. If both parents have the same rate of isoniazid inactivation, all of their children will have that rate. The results of the determination of antimicrobially active isoniazid and its metabolites in urine corresponded well with the results of isoniazid bioassay in the serum. The isoniazid serum concentrations could be elevated by giving higher doses of isoniazid or by adding PAS.—J. Haapanen

Antituberculous Drugs: 3-Iodo-Isonicotinic Acid (in Czech). K. Palát*, M. Čeladník, L. Nováček, and R. Urbančík. *Rozhl Tuberk*, October, 1959, 19: 716–719. (*University, Brno, Czechoslovakia)

3-Iodo-isonicotinic acid was prepared in the laboratory using 4-picoline as the basic material. Iodation of 4-picoline gave 3-iodo-4-picoline and, by oxidation of the latter, 3-iodo-isonicotinic acid was prepared. The antituberculous activity of a sodium salt of 3-iodo-isonicotinic acid was evaluated both *in vitro* and *in vivo*. The activity *in vitro* was rather disappointing. A concentration of 1γ per 1 ml. of media showed no tuberculostatic activity. Despite this, 3-iodo-isonicotinic acid was active *in vivo*. When it was administered subcutaneously to a group of mice, the average survival was 24.6 days, as compared with 29.8 days for equal doses of isoniazid and 19.7 days for the untreated control group.—J. Ilavsky

The Antimicrobial Activity of Kanamycin as Evaluated by Warburg's Microrespirometer (in Italian). S. Valenti*, M. Lucchesi, M. Zubiani, and B. Treves. *Ann Ist Forlanini*, No. 3, 1959, 19: 302–317. (*U. of Rome, Italy)

In order to evaluate the antimicrobial activity of kanamycin, 3 strains of Staphylococcus, 1 of *B. anthracis*, 4 of human *M. tuberculosis* (2 susceptible strains, 1 isoniazid resistant, and 1 streptomycin resistant), 1 bovine strain (BCG), and 1 paratuberculous strain (*M. phlei*), were grown in their own specific media. Suspensions of the different bacteria were added with various doses of kanamycin, kept at 37°C. for different periods of time, washed, and then studied with Warburg's microrespirometer to assess the oxygen consumption. The well-known wide-spectrum antimicrobial activity of kanamycin was confirmed, and its use in pulmonary diseases of mixed etiology is emphasized. The *in vitro* susceptibility to kanamycin seemed to change among different strains, even within the same species. Very low oxygen consumption was also found when bacteria had been in contact with high concentrations of the antimicrobial; it is therefore believed that some resistant cells may exist in the normal bacterial population.—I. Archetti

Some Considerations about Antimicrobial Resistance of *Mycobacterium Tuberculosis* (in Italian). F. Mandler*, G. Ganzetti, F. Basilico, R. Italia, and E. Corbella. *G Ital Tuberc*, No. 5, 1960, 14: 276–283. (*U. of Milan, Italy)

In order to estimate the frequency of antimicrobial resistance among the strains of *M. tuberculosis* isolated in a large hospital, the results obtained from a total of 3,093 bacteriologic examinations done during a 4-year period are given. A strain was considered resistant when at least ⅓ of the bacterial population in the control culture grew in the presence of the antimicrobial. At bacteriologic examination, 53.7 per cent of the cultures were found positive for *M. tuberculosis*; among those positive, 75.6 per cent were resistant, i.e., 47.8 per cent were resistant to streptomycin, 46.6 per cent to isoniazid, 6.5 per cent to PAS, and 22.9 per cent to cycloserine. The percentage of multiple resistant strains are also given. The characteristics of this resistance, from a quantitative point of view, were investigated, and it was found that strains were often resistant to a high concentration of the

antimicrobial; 26.3 per cent of the streptomycin-resistant bacilli were resistant to 250γ per ml., 28.0 per cent of the isoniazid-resistant bacilli were resistant to 100γ per ml., 32.5 per cent of the PAS-resistant bacilli were resistant to 50γ per ml., and 38.7 per cent of the cycloserine-resistant bacilli were resistant to 50γ per ml.—I. Archetti

Report on Forty-three Tuberculous Patients with either Pleural or Extrapleural Empyema Treated with Oleandomycin Associated with Tetracycline. Clinical and Laboratory Findings and Therapeutic Results (in Italian). G. D. Bianchera*, V. Gramazio, B. Occhialini, and E. Volpe. *Minerva Med*, No. 11, 1960, 51: 425–432. (*Villaggio Sanatoriale, Sondalo, Italy)

The treatment of chronic mixed empyema in the tuberculous patient has always been a difficult problem to solve. Forty-three male patients, 20 to 60 years of age, all but 1 with pulmonary tuberculosis and with either intrapleural or extrapleural empyema, were generally given sigmamycin (a drug consisting of a combination of tetracycline and oleandomycin) both orally (1 gm. daily) and *in loco* (150–500 mg. daily). The general conditions of the patients, who had been previously treated with other antimicrobials, were rather bad. Tolerance to the drug was good. The immediate results were considered excellent (18 per cent), good (53.6 per cent), moderate (25.6 per cent), and negative (2.5 per cent), as based on the general effects observed at the end of treatment. Among the factors which proved to be important for the results achieved were the type of bacilli associated with *M. tuberculosis*, the presence of a bronchopleural fistula or of a pleuroparietal drainage, the time interval between the occurrence of the empyema and the treatment, and the presence of tubercle bacilli in the fluid.—I. Archetti

A Contribution to the Problem of Possible Body Damage as a Result of Treatment with PAS and Antituberculous Drugs (in Czech). J. Štěpán* and V. Vorel. *Cas Lek Cesk*, January 22, 1960, 99: 111–117. (*U. of Plzeň, Czechoslovakia)

A few years ago, a tuberculous patient treated with massive doses of PAS died and a kidney affection was found at autopsy. This was interpreted as a nephrotic nephritis with an increased deposition of lipids. Alcohol extract of the kidney tissue contained increased levels of cholesterol and stenols. A similar condition was found at autopsy in a man who died in a traffic accident and who had been treated with PAS about 6 months prior to death. For this reason, the authors decided to study this problem. First, they systematically checked the kidneys of all patients who died who had been treated with PAS, and they compared the results with those who died in accidents. Second, biochemical changes in the plasma of patients were studied and compared with changes in the plasma of experimental animals. It was found that very often after administration of PAS a biochemical situation developed which is very much like that found in nephrotic nephritis. PAS alone does not produce these conditions. For this reason, the authors consider it very important to follow the renal function of patients and especially of those who previously suffered from renal diseases. Similarly, the liver and adrenal functions have to be followed during PAS and isoniazid therapy.—J. Ilavsky

Antibiotics and Terminal Pneumonia. A Postmortem Microbiological Study. Y. Kneeland* and K. M. Price. *Amer J Med*, December, 1960, 29: 967–979. (*Columbia U. College of Physicians and Surgeons, N. Y.)

This bacteriologic study of 201 autopsy reports was designed to determine what is being accomplished, with particular reference to pneumonia, by the current widespread use of antimicrobials. Past records in the Department of Pathology were compared with the reports of similar studies performed before the introduction of these relatively new therapeutic agents. Certain obvious conclusions were immediately apparent: "old-fashioned" lobar pneumonia as a cause of death has almost completely disappeared, and the organisms that were formerly considered of greatest significance in terminal pneumonia, all of which are uniformly susceptible to antimicrobials and do not tend to acquire resistance, have likewise withdrawn from the scene. These 3 organisms are the pneumococcus, the group A hemolytic Streptococcus, and *H. influenzae*. Their place has been taken by the Staphylococcus, which has become relatively more prominent than previously, and several gram-negative bacilli. Six organisms which seem to have taken over the role of terminal pulmonary pathogens are considered in some detail. In 26 patients, the pneumonia which contributed to death appeared to be due to a single one of these bacteria, and in only 5 instances was the patient receiving an antimicrobial to which the particular organism was susceptible. The correct antimicrobials will still be effective, even at the end stage of chronic disease. As there is obviously no way of determining, in a series of patients who have died, how often antimicrobials save life, the vexing question of the advisability of their widespread use cannot be answered from these data. Antimicrobials have modified the pattern, rather than the incidence, of terminal pneumonia.—T. H. Noehren

The Use of Prednisone (or Prednisolone) Associated with Antimicrobials in the Treatment of Severe Pulmonary Tuberculosis (in Italian). A. STAFFA*. *Ann Med Sondalo*, No. 4, 1960, 8: 246–266. (*Villaggio Sanatoriale, Sondalo, Italy)

Twenty-six patients with various forms of severe pulmonary tuberculosis, most of whom had already been treated with large doses of antimicrobials, were given daily from 5 to 20 mg. of either prednisone or prednisolone in association with specific and nonspecific antimicrobials. The results, based above all on objective improvement and roentgenographic examination, were encouraging. Clinical improvement was observed in 65.3 per cent of the patients, and in a few cases it was dramatic; this did not last long in 4 patients, 3 of whom had cases of silicotuberculosis. The best results were obtained in cases of early infiltrative nodular tuberculosis with a large exudative component; the improvement avoided chronicity of the processes, with the consequent surgery necessary. No untoward effects were observed. In only 1 case there was a worsening of the local symptomatology.—I. ARCHETTI

Deafness and Vestibular Impairment Caused by Strepto- and Dihydrostreptomycin. G. ROSSI*, E. CARANDO-DAMIANO, G. DE MICHELIS, and P. MAGGIOROTTI. *Acta Med Scand*, February, 1961, 169: 169–180. (*Turin U., Italy)

During the period from January 11, 1950, to December 31, 1959, there were 161 patients with hearing loss and 48 patients with vestibular impairment after treatment with dihydrostreptomycin (DHSM) or streptomycin (SM). Irreversible hearing loss occurred in 15 patients after administration of small doses of DHSM. Hearing loss due to DHSM was divided into 5 types of hearing disability: A—5 per cent; B—30 per cent; C—60 per cent; D—100 per cent, with threshold of hearing reaching 80–100 decibels only for the low tones; E—total deafness. An example of each type of loss is given, and the clinical characteristics and the consequences for the patients' social life are discussed.—E. DUNNER

On the Rectal Administration of Streptomycin (in Italian). M. RAMAGLIA* and A. MONTELLA. *Arch Tisiol*, No. 9, 1960, 15: 923–931. (*U. of Naples, Italy)

A study was made to learn if streptomycin was sufficiently adsorbed by the rectal mucosa, so that this route of administration could be used in a chronic disease such as tuberculosis. Twenty-four persons without any specific active process were given the antimicrobial in a dose of 0.25 or 0.5 gm.; 12 received the drug by the rectal route, and the other 12 intramuscularly. Samples of blood were taken 30 minutes, and 1, 2, and 4 hours after the administration. The results showed that a sufficient concentration of the drug in the blood was not obtained by the rectal route, and use of the method in the treatment of tuberculosis was therefore not justified.—I. ARCHETTI

The Medical Letter*. (*136 E. 57th St., N. Y.)

May 26, 1961, 3: 41–44: The anabolic-androgenic steroids. A complex of adrenochrome monosemicarbazone and sodium salicylate (Adrenosem Salicylate®) and hemostasis. Aminopyrine nonprescription drugs abroad.

June 9, 1961, 3: 45–48: Sulfonamide mixtures and combinations. Tests of Digitoxin tablets. Thist®.

PULMONARY PHYSIOLOGY

Apnea, Suction, and Hyperventilation: Effect on Arterial Oxygen Saturation. J. DOWNES*, J. WILSON, and D. GOODSON. *Anesthesiology*, January–February, 1961, 22: 29–33. (*141 Fairfax Road, Rosemont, Pa.)

A study was undertaken to determine the amount of arterial desaturation incurred by patients anesthetized with nitrous oxide and oxygen when subjected to 1-minute periods of apnea both with and without prior hyperventilation with oxygen. The effect on arterial oxygen saturation of endotracheal suction during apnea is also studied. Eleven adult patients undergoing pulmonary resection were the subjects of the present study. The methods and results are presented in detail. A Wood's single-scale direct-reading ear oximeter was used. One minute of apnea resulted in arterial desaturation to or below 93 per cent in ¾ of the trials. Hyperventilation with oxygen for 15 seconds prior to apnea caused arterial saturation to remain above 95 per cent in every patient during 2 minutes of apnea with intact pleura and 1 minute of apnea with open pleura. Endotracheal suction during apnea did not significantly change arterial oxygen saturation. Desaturation was greater with the pleura open. When arterial oxygen saturation falls to 90 to 93 per cent, hypoxia can occur rapidly. The data suggest that a 15-second interval of hyperventilation with oxygen should precede all periods of planned apnea.—E. SHABART

Does Respiratory Acidosis Cause "Cyclopropane Hypertension." T. LI* and B. ETSTEN. *Anesthesiology*, January–February, 1961, 22: 49–55. (*Tufts U. School of Med., Boston, Mass.)

The elevation of arterial blood pressure, right atrial pressure, central venous pressure, and pulmonary arterial pressure justifies the introduction

of the term "cyclopropane hypertension." Twenty patients were studied prior to surgery. The methods of study and the results are described in detail. An increase in arterial pressure is not significantly related to a decrease in alveolar ventilation during surgical levels of cyclopropane anesthesia. Arterial pressure increase occurs as frequently during normal levels of arterial PCO_2 as during elevated levels. No significant difference was found in arterial blood pressure, heart rate, total peripheral resistance, cardiac index, stroke volume, mean circulation time, intrathoracic blood volume, and left ventricular work between the normal and high levels of PCO_2. The study concludes that cyclopropane hypertension is not related to respiratory acidosis by hypoventilation.
—E. SHABART

Pulmonary Function in the Hamman-Rich Syndrome. The Abnormalities of Ventilation, Blood Gases and Diffusion at Rest and on Exercise. R. A. B. HOLLAND* and R. B. BLACKET. *Amer J Med,* December, 1960, 29: 955–966. (*U. of Sidney, New South Wales, Australia)

Pulmonary function was studied in 5 patients with the Hamman-Rich syndrome. The spirometric tests showed a reduction in lung volume with no subdivision particularly affected; the maximal breathing capacity varied, being normal or reduced. The increased ventilation was due largely to an increase in the physiologic dead space; the alveolar ventilation at rest was usually normal. The D_{co} (Filley method) was greatly lowered in all patients at rest and on exercise. The lowering accounts for some of the arterial oxygen unsaturation in severe cases and, in general, correlates well with the severity. Resting arterial oxygen saturation was normal or reduced, depending on the severity of the case. In all cases the saturation fell on exercise. There was no consistent pattern of blood carbon dioxide. In no case was the diffusion defect great enough to account for a marked reduction of arterial oxygen saturation; when this was present there was an increase in venous admixture. Impaired diffusion, increased physiologic dead space, and increased venous admixture all play a part in the causation of the disability in the Hamman-Rich syndrome.—T. H. NOEHREN

Heat and Water Exchange in the Respiratory Tract. J. E. WALKER* and R. E. WELLS, JR. *Amer J Med,* February, 1961, 30: 259–267. (*Harvard Med. School, Boston, Mass.)

The respiratory system relies on an elegant set of defense mechanisms to protect its internal environment. Besides filtering foreign matter and bacteria, the upper respiratory tract is able to warm and saturate inspired air so that in climates as cold as −100°C., air entering the alveoli has attained body temperature and is saturated with water vapor. The ability of the upper respiratory tract to "condition" inspired air in this manner not only protects the alveolar membrane from thermal injury but also permits the vital process of carbon dioxide excretion to occur with such rapidity by keeping the membrane wet. This most important function of the upper respiratory tract is accounted for by the rapid exchange of heat and water between respired air and the respiratory tract mucosa. This exchange occurs with amazing speed in both directions; during inspiration air is warmed and humidified for the alveolar environment, and during expiration heat and water are reclaimed by the mucosa. The dual ability of the respiratory tract to condition air during inspiration and to conserve heat and water during expiration is based upon physical principles of heat and water exchange that are well known to the physicist and engineer, but are infrequently utilized by the physician. The present report is an attempt to translate into clear terms the basic principles of heat and water exchange as they pertain to this "air conditioning" function of the normal respiratory tract. The concepts gained through an understanding of these principles are then applied to a number of clinical problems involving the respiratory tract in disease.—T. H. NOEHREN

Abnormalities of Pulmonary Gas Exchange in Obesity. S. I. SAID*. *Ann Intern Med,* December, 1960, 53: 1121–1129. (*Med. College of Virginia, Richmond)

Pulmonary gas exchange was studied in 20 obese subjects. All showed some degree of reduction in arterial oxygen tension. Since hypoventilation (CO_2 retention) was present in only 8 of the subjects, it is evident that the mechanism of arterial hypoxia was not always on the basis of hypoventilation. Furthermore, even in the subjects who exhibited CO_2 retention, the degree of arterial deoxygenation was, in all but 1, greater than could be accounted for simply by the elevated arterial CO_2 tension. The pulmonary diffusing capacity was not reduced sufficiently to account for the abnormally low arterial oxygen tension present in the resting condition. Since the venous admixture and physiologic dead space ratios were abnormally elevated, the conclusion is suggested that the basic disorder in gas exchange in cases of obesity is one of distribution, that is, a disturbance in ventilation-perfusion relationships. It is possible that the shallow breathing of the obese subject at a small lung volume causes small airway closure and regional atelectasis, which lead to increased venous admixture. It is also possible that shallow and irregular breathing deprives the pulmonary capillary

circulation of the important pumping action by which normal respiratory movement promotes uniformity of capillary perfusion. Uneven perfusion of alveoli will result in a disturbed ventilation-perfusion ratio, with an increase in physiologic dead space and physiologic shunt. It is possible that CO_2 retention is a relatively late development in the evolution of respiratory dysfunction due to obesity. —T. H. Noehren

The Effects of Intermittent Positive Pressure Breathing on the Intrapulmonary Distribution of Inspired Air. G. Torres*, H. A. Lyons, and P. Emerson. *Amer J Med*, December, 1960, 29: 946–954. (*State U. of N. Y., Brooklyn)

The effect of intermittent positive pressure breathing on the intrapulmonary mixing of inspired gas was studied in 12 normal subjects and 9 emphysematous patients by the nitrogen washout method. The evenness of ventilation as measured by Fowler's percentage of lung clearance delay was improved in the normal subjects and, to an even greater degree, in the emphysematous patients. In all of the normal subjects and in those emphysematous patients who could do so, a voluntary increase in tidal volume comparable to that produced by intermittent positive pressure breathing effected the changes in the over-all efficiency of ventilation. It is concluded that the beneficial effects of intermittent positive pressure breathing on the intrapulmonary mixing of gases are due to the increase in tidal volume obtained, and they are of particular value in those patients who are unable either to maintain an effective tidal volume or increase it.—T. H. Noehren

The VUT Open Circuit Electronic Spirograph (in Czech). B. Dubsky*, H. Felkel, R. Feuereisl, and O. Straka. *Rozhl Tuberk*, February, 1959, 19: 124–133. (*Tuberculosis Research Institute, Prague, Czechoslovakia)

A laboratory model of an electronic spirograph with an open circuit is described. This apparatus was constructed on principles entirely different from commonly used mechanical spirographs. It measures electrically the volumes of inhaled and exhaled air as well as the percentage of carbon dioxide. The spirographic record has the usual pattern. The apparatus is small and light and can be easily carried by one person.—J. Ilavsky

Preliminary Notes on the Action of Strychnine upon Pulmonary Ventilation in Man (in Czech). J. Skládal*, V. Hons, and B. Kočí. *Cas Lek Cesk*, April 29, 1960, 99: 570–571. (*Charles U., Prague, Czechoslovakia)

It is known that strychnine in higher doses stimulates the respiratory and vasomotor centra, but there are no reports dealing with the effects of small doses of strychnine, which are active mainly upon the spinal cord. The authors studied the mechanism and origin of the typical curve of diaphragmatic motion. When small doses of strychnine were applied in a dyspneic person, the dyspnea was considerably alleviated or disappeared completely. The transition from dyspnea to eupnea started approximately 7 minutes after the injection of strychnine, and the maximal effect was reached in about 30 minutes. Euphoria and improved breathing lasted for 1 to 5 days. This favorable effect was achieved with doses of 1 to 2 mg. of strychnine nitrate. The majority of persons used in these experiments were patients with either rather mild dyspnea due to ventilation insufficiency combined with nervous lability, or not-too-severe emphysema. The strychnine eupnea was accompanied by increased amplitude of diaphragmatic motion which was often clearly visible without any aid.—J. Ilavsky

The Anatomic and Functional Capacity of Substitution of the Lung (in Italian). G. P. Fioretti*, E. Fiorelli, G. D'Alfonso, E. Catena, and T. Tuzi. *Arch Tisiol*, No. 9, 1960, 15: 811–876. (*U. of Naples, Italy)

I. Bronchographic Data: 811–834.

In an investigation of the actual capacity of the lung to substitute anatomically and functionally for a lacking part of the organ, lobectomy was performed on 36 puppies. Twelve died following an epidemic of distemper. Roentgenographic and bronchographic examinations were performed before and after the operation, and at different intervals up to 12 months. Two stages must be considered after resection: (*1*) Adaptation, due to the new static condition of the thorax; this is rapid and complete in lower lobectomy, and the result is an almost normal picture of the thorax and lung. In upper lobectomy a slight retraction of the thorax was frequently found, which was of greater degree in concomitant lower and middle lobectomy. A change in location and direction could be observed in the bronchial branches, as a first response to the new situation. (*2*) As the animals grew, bronchography disclosed that the branches were becoming progressively larger, with a diameter and morphologic aspect very similar to those of the contralateral normal lung.

II. Pathologic Picture: 835–868.

Twenty puppies of the 36 submitted to lobectomy were killed, and gross anatomic and microscopic examinations of the thorax and lung were performed. Bronchographic studies and measurements of the broncho-alveolar structures were also carried out. The results are given in detail, with

the conclusion that, after the resection of a lung lobe, the remaining lobe grew so as to almost reach the size of a contralateral normal lung. No changes were observed in the first days after operation, except for congestion and hyperemia. The residual lobe began to grow about the 23rd day after the resection and continued to grow until about the 43rd day, at which time the entire hemithorax was occupied by the organ. This was clearly evident in the animals which had lower lobectomy, and was less obvious in those with either upper or double lobectomy. Initially, the bronchial tree lengthened, and reduction occurred in the lumina of the various branches; following adaptation, the bronchographic aspect was that of a normal bronchial leafage. All of the stages are described histologically. When the animals were killed at later stages, the structural picture of the lung and alveoli, and the number of alveoli per square millimeter, seemed to be very similar to those of the contralateral normal lung. A significant increase in the bronchoalveolar structures was found.

III. Functional Data: 869–876.

Observations were made on 5 puppies, 30 to 50 days of age, that had been treated with lobectomy; 2 had the procedure performed on the lower left lobe, 2 on the lower right, and 1 had upper left lobectomy. Bronchospirographic examination was made 11 to 12 months after surgery, using a Carlens tube and a Dargatz bronchospirograph, type 198. The values of the various functional indices are given. The conclusions of the study are that the lobectomized lung functionally substitutes for the resected portion, and that it contributes to the respiratory function both quantitatively and qualitatively in a manner similar to the normal contralateral lung.—I. Archetti

Quantitative Retention of Tobacco Smoke on Membrane Filters and Estimation of Its Retention in the Lung (in Czech). M. Polydorová*. *Rozhl Tuberk,* September, 1959, 19: 607–611. (*Academy of Sciences, Prague, Czechoslovakia)

A relatively simple method was designed and used in this experimental study. Membrane filters were used and were found satisfactory. It was demonstrated that medium-heavy smokers retain more than 80 per cent of the smoke in their lungs. The percentage of smoke retained in moderate smokers was much lower.—J. Ilavsky

MICROBIOLOGY AND IMMUNOLOGY

Enzymic Degradation of Adenosine and Inosine Phosphates in Mycobacterial Lysates (in Italian). P. L. Ipata*, M. C. Gabrielli, and C. Cattaneo. *Ann Ist Forlanini,* No. 4, 1959, 19: 427–438. (*U. of Rome, Italy)

In a study of the enzymes of mycobacteria concerned with the metabolism of adenosine and inosine phosphates, various enzymic activities were taken into consideration. The mycobacteria used were human, bovine, or avian strains grown in Sauton medium. The bacterial cells were disintegrated in a special apparatus designed by Zampieri, and centrifuged at 15,000 times gravity. Three fractions were obtained: the total lysate, the soluble fraction, and the sediment. The substrates were chromatographically pure; the experiments were done in barbital (Veronal®) buffer at different ranges of pH. The final products were identified by the chromatographic procedure of Cerletti *et al.* No enzymic activity was detected in the sediment, while both the soluble fraction and the lysate were found to contain the following enzymic systems: ATPase, ADPase, Adenosine phosphokinase, ITPase, IDPase, and As-deaminase. No evidence was obtained of dephosphorylation of the tested mononucleotides (i.e., 5-adenylic acid (AMP®), IMP, and UMP), or of nucleosidase activities occurring in the case of the Is and AS substrates. No difference in the extent of dephosphorylase and adenosine phosphokinase activities was observed among the various types of mycobacteria used in this study; deamination of As was found to be less marked in the human strains.
—I. Archetti

Lipids of *Mycobacterium Tuberculosis*, var. *Bovis*—BCG (in Czech). J. Pokorný* and V. Šalanská. *Rozhl Tuberk,* April, 1960, 20: 264–272. (*Tuberculosis Research Institute, Prague, Czechoslovakia)

The lipid content was studied of 11 strains of BCG used in the preparation of vaccines against tuberculosis in Brazil, China, Czechoslovakia, Denmark, England, France, Germany, Japan, Poland, and the Soviet Union. It was found that the lipid content in a BCG culture depends upon the composition of the culture media, especially the amount of glycerol. Mycobacterial lipids exert a basic influence upon the chemotactic and immunogenic properties of vaccines. Their composition depends upon the character and degree of local reaction as well as the development of immunity. Individual BCG substrains differ considerably in their lipid composition and higher fatty-acid content. This unequal chemical composition explains variations in the quality of various vaccines, especially variations in their residual virulence.—J. Ilavsky

Studies on Primary Atypical Pneumonia. III. A Factor in Normal Serum which Enhances the Reaction between PAP Virus and Convalescent Serum. C. Liu*. *J Exp Med,* No. 1, 1961, 113: 111–123. (*U. of Kansas School of Med., Kansas City)

A factor present in fresh normal or guinea pig serum enhances the primary atypical pneumonia (PAP) antibody titers in serologic reaction with its homologous virus as tested by indirect fluorescent antibody staining. This factor is not properdin. It has many properties similar to complement, but may not be identical with complement. The addition of this enhancement factor to human sera convalescent from primary atypical pneumonia has made the serologic test more sensitive without affecting its specificity (Author's summary).—C. H. PIERCE

Influence of Antituberculous Drugs upon the Metabolism of Phosphorus and Iron in Mycobacteria (in Czech). M. POLSTER*. *Rozhl Tuberk*, June, 1960, 20: 460–463. (*U. of Brno, Czechoslovakia)

Radioactive phosphorus and iron were used in the present studies. It was observed that bacteriostatic concentrations of isoniazid and PAS caused an accumulation of phosphorus in the cells of saphrophytic mycobacteria, but there was no accumulation of iron. The author believes that isoniazid and PAS do not disturb the oxidation-reduction reactions leading to the formation of substances containing macroergic phosphate (for example, adenosine triphosphate, phosphenol pyruvic acid, acetylphosphate, and others), and that isoniazid and PAS prevent the decomposition of these substances, resulting in the accumulation of phosphate.—J. ILAVSKY

Significance of Rat Blood for Differentiation of Human and Bovine Mycobacteria (in Czech). M. MATĚJKA*. *Rozhl Tuberk*, May, 1960, 20: 388–390. (*Krajská Hyg. Epidemiol. Stanice, České Budějovice, Czechoslovakia)

The author tried to develop a method for differentiation of the human and bovine varieties of *M. tuberculosis*. The work of Hinglais and Langlande and their report on differences in the growth of mycobacteria when cultured on agar containing 20 per cent human blood, rabbit blood, or rat blood served as a basis for the present study. The author tested and compared 119 different strains of myobacteria before reaching the conclusion that this method has no practical value. The growth of human mycobacteria on rat blood agar was demonstrable even when a minimal inoculum was used. Saprophytic mycobacteria showed a little more inhibition on rat blood agar, but even these strains grew.—J. ILAVSKY

A New Method of Determining the Susceptibility of *M. Tuberculosis* to Antimicrobials. A Method Utilizing Serum (in French). J. AUDRIX*. *Rev Tuberc (Par)*, November, 1960, 24: 1214–1220. (*Briancon, France)

The usual *in vitro* methods of susceptibility testing can be said to be inapplicable to conditions *in vivo* and in contradiction to clinical findings. The authors have devised a new method for the preparation of culture media which utilizes the serum of patients under chemotherapy: after cessation of chemotherapy for at least 48 hours, the drug to be studied is injected intramuscularly (500 mg. of isoniazid or 1.0 gm. of streptomycin); 2 hours after injection blood is drawn, and the serum is diluted with beef serum in various proportions. Liquid Jensen medium is added. The final medium contains 17 per cent serum with isoniazid and 50 per cent serum with dihydrostreptomycin. These media are inoculated with a susceptible control strain such as H37Rv or 1099IP and with the strain of the patient. Readings are done after 10 and 20 days. The results are expressed as a relation between the dilution of the last tube showing growth of the susceptible strain to the dilution of the last tube showing growth with this strain of the patient.—V. LEITES

EXPERIMENTAL PATHOLOGY

Further Observations on the Pathogenesis of Plate-like Atelectasis (in German). GY. PETRÁNYI*. *Z Tuberk*, October, 1960, 116: 50–62. (*Sanatorium "Pro Juventute," Hárshegy, Budapest, Hungary)

Nine patients with plate-like atelectasis were examined thoroughly by bronchoscopy, thoracoscopy, and serial roentgenograms. The ages ranged from 6 to 17 years. The alveolar collapse was caused by a pathologic neuromuscular or neurovascular functioning of the corresponding part of the lung. The preferred location was the intersegmental area. Atelectasis was also found in the upper lobes, where the site of predilection was the borderline between the apical and anterior segments.
—J. HAAPANEN

Behavior of the Serum Proteins in Primary Cancer of the Lung (in Italian). R. VIRGILIO*. *Arch Tisiol*, No. 11, 1960, 15: 1103–1116. (*U. of Naples, Italy)

The serum protein content was analyzed in 23 patients with primary cancer of the lung; the diagnosis was confirmed by histologic examinations. Of a total of 26 analyses, the protein content was found to be less than normal in 14 instances, with a mean value of 6.10 gm. per 100 ml. (the minimum was 4.20 gm. per 100 ml. and the maximum was 6.90 gm. per 100 ml.). The albumin-globulin ratio was always reversed, with a mean value of 0.57, both in patients with normal or hypoproteinemia. The electrophoretic pattern was characterized by a statistically significant decrease

of albumin and an increase of the alpha and gamma"-globulins; the patients with hypoproteinemia showed a change only in alpha globulin. It is stressed that the variations observed in these cases are not specific for cancer of the lung, and that therefore they do not have any significance in the diagnosis of the disease.—I. Archetti

I. The Blood Catalase in Infection with *M. Tuberculosis*. II. The Liver Catalase in Infection with Catalase-positive and Catalase-negative Tubercle Bacilli and the Effect of Catalase Inhibitors (in German). H. Heller*. *Beitr Klin Tuberk*, August, 1960, 122: 377–404. (*Medizinische Universitäteklinik, Halle a.d.S., East Germany)

Investigations revealed a correlation between the virulence of the *M. tuberculosis* and its catalase activity. Using 4- to 6-week-old Löwenstein cultures and freshly prepared 3 per cent hydrogen dioxide solution, the following qualitative catalase values were demonstrated in 4 strains of mycobacteria: BCG +; Mandrill strain ++; H37Rv +++; the isoniazid-resistant tubercle bacillus was catalase negative. By quantitative determinations, the values were greater when the examination was repeated several months later. The cause of this increase is the fact that the catalase remains active for a long time after the death of the bacterium; however, since the endocatalase becomes free only after the bacterium dies, its activity added to the regular ectocatalase accounted for the increase. There is no report in the literature dealing with blood catalase activity in connection with tuberculous infection. As a basis for such investigation, catalase determinations were done in the blood of nontuberculous horses, dogs, cattle, and pigs, as well as the usual laboratory animals. In addition, the catalase index (the amount of catalase per erythrocyte) was also determined. (The catalase index showed normal values (75–150) in 53 patients with far advanced tuberculosis in various stages with and without isoniazid treatment.) The investigation lasted for 1½ years, and at least 20 animals of each species, both females and males, were used. The examinations were carried out with the 4 different strains. The method of investigation and the results obtained in each animal species are reported in detail and illustrated graphically. Since there is no known suitable chromogenic substrate, histochemical testing of catalase is not possible as yet. Larger animals had a relatively higher blood catalase level, and its value was not influenced by the sex or age of the animals. Without exception, the quick animals (horses, mice, rats, rabbits, cats, and dogs) had low catalase levels, increased resistance to the disease, and a productive course of tuberculosis, as opposed to the slow animals (such as guinea pigs, cattle, and pigs) which had high blood catalase levels; e.g., the guinea pig with the highest blood catalase level is the animal most susceptible to tuberculosis. The type of noxa is also important: the pig develops exudative disease following an infection by bovine mycobacteria, and productive process from the avian tubercle bacillus.

In another series of experiments, the various animal species were infected with the same 4 strains of mycobacteria and treated with isoniazid. Noninfected animals served as controls. In all of the animals, the blood catalase had increased after 14 days of daily administration of isoniazid; the values were different in each species, and they varied also according to the isoniazid dosage employed. The possible connection between blood catalase (a hemin protein with a molecular weight of 225,000 containing 0.09 per cent iron) and the siderocytes was also investigated. First the number of siderocytes was determined in the blood of healthy animals. Then they were infected with H37Rv tubercle bacilli. There was no change in the number of siderocytes in mice and rats, a slight elevation was noted in rabbits, and a considerable increase in guinea pigs. After treatment with hydroxylamine, a catalase inhibitor, the number of siderocytes remained unchanged in the rabbits but almost completely disappeared in the other animals. It is known that virulent tubercle bacilli are rich in lipoids, in contrast to the avirulent organisms. Comparing the lipoid values with those of the blood catalase, animals with high lipase levels had decreased and those with low lipase levels had an increased blood catalase activity. The catalase reaction is suitable for separating catalase-negative, isoniazid-resistant mycobacteria from a definite population of tubercle bacilli; however, no absolute differentiation is possible. The blood catalase level may have some connection with the tuberculous process, but it does not influence its course. As a diagnostic test, the blood catalase determination is of no importance.

The liver catalase was examined in mice, rats, rabbits, pigs, guinea pigs, and cattle. Its amount varied according to the species but was not influenced by the sex of the animal. When the animals were infected with isoniazid-resistant tubercle bacilli or BCG, the liver catalase remained normal even when the blood catalase values increased. The results were not conclusive after infection with the H37Rv or Mandrill strains. The blood and liver catalase examinations were carried out with the same 4 strains of mycobacteria in the various animals with reference to the effects of hydroxylamine and isoniazid treatment. The results obtained were different in the various animals and not conducive to generalization. Comparing the

changes in the blood catalase level, the siderocytes, and the liver catalase level in the various animal species under normal conditions and after infection with the 4 strains, it is suggested that the considerable variations in the blood catalase level may be produced not only by the administration of certain drugs but also by infection with various noxious agents.—Z. Virágh

Studies on the Bronchodilating Action of Ganglioplegic and Vagolytic Drugs in Emphysematous and Silicotic Patients with Asthmatic Bronchitis (in Italian). E. Sartonelli* and P. G. Scotti. *Med Lavoro*, No. 3, 1960, 51: 174–182. (*U. of Milan, Italy)

The bronchodilating action of 2 vagolytic drugs (diphemanil methylsulfate and dicyclomine hydrochloride) and 3 ganglioplegic drugs (promazine, chlorpromazine, and levomepromazine), given by different routes (intravenous, intramuscular, and aerosol), was studied in 34 patients with chronic asthmatic bronchitis associated with pulmonary emphysema of various degrees, and in 20 patients who had silicosis of varying severity complicating the bronchitis. The effect of the substances was evaluated by spirographic tests using Knipping's spirograph model 157. Proper control studies were done in each of the patients with epinephrine, aminophylline, and isoproterenol. Both vagolytic drugs had a bronchodilating action and did not cause, under the conditions of this experiment, any untoward symptoms. The 3 ganglioplegic drugs also showed a bronchodilating activity which was particularly evident when they were administered endovenously; chlorpromazine was always badly tolerated by this route. The usefulness of these drugs in the treatment of bronchospastic syndromes is discussed.—I. Archetti

Influence of Isoniazid and PAS upon the Level of Ascorbic Acid and Cholesterol in the Suprarenal Glands of Rats (in Czech). M. Kohoutek* and R. Krulík. *Rozhl Tuberk*, April, 1960, 20: 286–289. (*Tuberculosis Research Institute, Prague, Czechoslovakia)

In the first group of experiments, 50 mg. of isoniazid a day were administered perorally for 14 days. In the second group, 1 gm. of PAS was administered daily; the control group received physiologic saline solution. It was found that the level of ascorbic acid and the level of cholesterol in rats started decreasing the day following the first dose and continued to decrease for 14 days, after which the experiments were terminated. Simultaneously, the average weight of the suprarenal glands increased. The authors consider the decrease of the ascorbic acid and cholesterol levels and the increase in weight of the suprarenal glands as proof of suprarenal gland stimulation.—J. Ilavsky

Effect of Lipopolysaccharide Extract from *Salmonella Abortus Equine* upon the Evolution of Tuberculous Cavities Resistant to Antibacillary Medication (in French). G. Favez* and V. Tatti. *Rev Bras Tuberc*, December, 1960, 206: 45–47. (*U. of Lausanne, Switzerland)

Of a group of 432 tuberculous patients who were admitted to the Tuberculosis Service of the University Hospital and submitted to identical treatment, 29 patients who had presented cavities showed a quiescent state in the healing process on roentgenographic observation. These patients had originally shown initial improvement. This state of inactivity in the healing process was observed between the third and fifth months of treatment. The administration of the extract from *Salmonella abortus equine* accelerated the healing process toward cavity closure.—L. de Mello

Antituberculous Activity of Milk. Isolation and Concentration of Active Substances (in Czech). Z. Franc*, O. Horešovský, V. Bydžovsky, A. Čížková, and I. M. Hais. *Rozhl Tuberk*, June, 1959, 19: 475–480. (*Pharmacologic and Biologic Research Institute, Prague, Czechoslovakia)

Previous findings that cow's milk has a demonstrable inhibiting effect upon experimental tuberculosis in mice were re-examined. The technique of isolation and concentration of active substances was attempted. Fresh milk was acidified, the casein removed, and the whey dialyzed. The active substances passed through the cellophane membrane and were removed from dialysate by adsorption on charcoal. They were eluted from charcoal with acetic acid and water, precipitated with acetone, and the sediment was lyophilized. When this material was analyzed, various sugars, amino acids, fatty acids, and many unidentified substances were found.—J. Ilavsky

Vaccination of Guinea Pigs with Live BCG Labeled with Radioiodine (I^{131}) (in Czech). L. Trnka*, J. Pokorný, and J. Liebster. *Rozhl Tuberk*, April, 1958, 19: 288–290. (*Výzkumný ústav tuberkulósy, Prague, Czechoslovakia)

Living BCG bacilli were labeled with radioactive iodine (I^{131}). After experimental intradermal inoculation into guinea pigs, the BCG were rapidly transported from the site of injection to the internal organs through the lymphatic routes. After a single oral dose, only a small fraction penetrated into the lymphatic system and internal organs of the guinea pigs, some of the mycobacteria

being absorbed by the mucous membrane of the upper part of the digestive tract.—J. Ilavsky

Changes in the Serum Content of Iron and Transferrin in Patients with Pulmonary Tuberculosis, not Treated with Antimicrobials (in Italian). C. Cattaneo*, U. Fantoli, and L. Belasio. *Ann Ist Forlanini*, No. 3, 1959, 19: 332–348. (*U. of Rome, Italy)

The behavior of the iron and transferrin serum content was investigated in a study of anemia in tuberculosis. Schade's method was used in 97 patients (43 males and 54 females, with a mean age of 38 years) with pulmonary tuberculosis who had never been treated with antimicrobials. A decrease in the iron and transferrin content was frequently found and was more evident in the exudative and active forms of the disease. These results were generally, but not always, parallel, proving once more that hypotransferrinemia has an important role in the genesis of hyposideremia, but is only one of the factors causing such a condition.
—I. Archetti

Sideremia and Transferrinemia in Osteoarticular Tuberculosis (in Italian). U. Fantoli*, G. Ciniglio, and L. Belasio. *Ann Ist Forlanini*, No. 4, 1959, 19: 467–478. (*U. of Rome, Italy)

This second paper of a series on anemia in tuberculosis presents the results obtained in 58 patients with osteoarticular tuberculosis; 22 were children (mean age, 9 years) and 36, adults. Schade's method was again employed. The same changes as those observed in pulmonary tuberculosis were found, except that they were minor and inconstant. The hyposideremia was not related to either the activity or the evolution of the specific process. It was more frequently found in cases of osseous colliquation with formation of an ossifluent abscess, perhaps because of a more intense participation of the entire organism in the process. The hyposideremia was not always associated with a reduced binding capacity of the serum for iron.—I. Archetti

The Histogenesis of Experimental Silicosis. I. Methods for the Histologic Evaluation of Experimentally Induced Dust Lesions. U. Saffiotti*. *Med. Lavoro*, No. 1, 1960, 51: 10–17. (*U. of Milan, Italy)

A new classification is given for lesions experimentally induced by fibrogenic dust. It is based upon an evaluation of the extent of the lesions both in the lung and in the lymph nodes (from grade I to grade IV), and the occurrence of certain types of cellular elements (histiocytes, macrophages with severe necrotic changes, plasma cells, mast cells, fibroblasts, neutrophils, eosinophils, and lymphocytes) in the tissue reaction (from grade I to grade V). A similar graded classification is given for fibrosis and hyalinosis.

II. Cellular and Tissue Reactions in the Histogenesis of Pulmonary Lesions. U. Saffiotti, A. Tommasini Degna, and L. Mayer. *Med Lavoro*, No. 8–9, 1960, 51: 518–552.

The results are given of a study undertaken to follow up a detailed analysis of the cellular and tissue elements which play a role in the histogenesis of experimental silicosis. Five groups of 22 rats each were given by intratracheal injection a single dose (50 mg. in 0.5 ml.) of a sterile dust suspension of hematite, quartz, cristobalite, and tridymite. The animals were killed at different intervals after the onset of the experiment, from 7 days to 6 months, and the lungs and lymph nodes were prepared for histologic examination and stained by various methods. The sections were observed both in ordinary and under polarized light in order to localize the dust particles. All of the sections were examined, and the lesions which were found were classified according to the technique described in the preceding paper. The gross appearance and the histologic findings in the various groups, including the controls, are given in detail. From the data obtained in this study it seems that the main phases of cellular and tissue reactions to the dusts followed a constant pattern, i.e., phagocytosis, extensive necrosis of dust-laden macrophages, proliferation of histiocytes and fibroblasts, intense plasmacellular reaction, proliferation and degranulation of mast cells, and the appearance of a network of collagen fibers with formation of hyaline substance and, subsequently, fibrohyaline nodules.
—I. Archetti

Tuberculostatic Effect of Bee Propolis in Guinea Pig Experiments (in Czech). J. Šulová* and V. Šalanská. *Rozhl Tuberk*, June, 1959, 19: 472–474. (*Tuberculosis Research Institute, Prague, Czechoslovakia)

A water extract of bee propolis was prepared. Thirty subcutaneously infected guinea pigs were divided into 3 groups of 10 animals each. One group was treated for 82 days with 25 mg. of the extract per guinea pig. The second group was treated with the same dose injected subcutaneously for the same length of time. The third group was left as an untreated control group. Treatment did not prevent the development of tuberculous lesions, and the lesions were only slightly smaller in treated groups.—J. Ilavsky

Vascular and Connective Tissue Substratum and Histopathogenesis of Tuberculous Lung Lesions. II. Nodular Infiltrates (in Italian). Q. Lunetta*. *Ann Med Sondalo*, No. 4, 1960, 9: 217–231. (*Villaggio Sanatoriale, Sondalo, Italy)

Thirty-four circumscribed, nodular, tuberculous infiltrates were studied both histologically and histochemically in material obtained from 22 cases of either lobar or segmental lung resection performed for lesions of different types which had been already treated with antimicrobials. All of the nodules had a complete capsule, generally with a hyaline layer in contact with the caseum. Cells were rather scanty in the capsule, and were more numerous and of an histiocytic nature in the hyaline layer; vascular structures were rare. When the nodule showed colliquation, the pattern was different because of an increasing invasion of the capsule by infiltrative cells and more numerous capillaries. According to the histologic and histochemical characteristics of the structure of the caseous process, two types of nodules could be distinguished: a more frequent one (76.5 per cent) with an homogeneous aspect, in which the outlines of the old structure were still observable; the second type, very similar to tuberculoma, was formed by a group of caseous nodules partially separated by an abundant collagenous hyperplasia. In one case of the first group the "crescent-shaped cells" described by the author in 1958 were observed in the walls of the pulmonary tuberculous cavities. Perifocal lesions were always present, but to a very limited extent.—I. Archetti

PUBLIC HEALTH AND EPIDEMIOLOGY

Serial Tuberculin Testing of Young Children in a London Borough. J. A. Keeping* and R. Cruickshank. *Tubercle*, August, 1960, 41: 239–246. (*St. Mary's Hosp. Med. School, London, England)

An investigation was carried out on the tuberculin status of 4,833 children aged 0–5 years in Paddington, a London borough with an area of 1,357 acres. The population had fallen from 124,800 in 1952 to 115,700 in 1958, largely as the result of rehousing out of London and the replacement of slum property by modern blocks of flats. Nevertheless, housing conditions are still poor, and overcrowding is common. The population is comprised of many different nationalities: southern Irish, Poles, Austrians, Germans, Indians, West Indians, and Africans. The proportion of positive reactors to the tuberculin jelly test increased from 0.4 per cent at 2 years to 1.9 per cent at 5 years. Fifty-four per cent of infected children showed clinical or roentgenographic evidence of disease, but there were no deaths from tuberculosis during the period of the investigation. A source of infection was found for 60 per cent of the positive reactors, and 2 children with miliary and meningeal tuberculosis were in close contact with active adult tuberculosis. There was no demonstrable relationship between evidence of disease other than the positive tuberculin test and the social class involved. The jelly test emerged as a reliable index of the presence of tuberculin sensitivity in this age group, giving no false-negative and only 0.5 per cent false-positive reactions when compared with the 10-TU Mantoux test. However, the Heaf multiple-puncture test has advantages over the jelly test in tuberculin surveys in young children.—M. J. Small

Further Studies on the Potency of the Purified Protein Derivatives of Tuberculin (PPD-s). II. Comparison of the Potencies of the WHO PPD and the Japanese 1:2,000 Dilution of Old Tuberculin (in Japanese). D. Maeda*, N. Asami, and T. Murohashi. *Kekkaku*, September, 1960, 35: 618–621. (*National Institute of Health, Tokyo, Japan)

Although the method of preparation of PPD and PPD-s is generally the same in principle everywhere, the potency of each product is best determined by analyzing the results of field work on a sizeable sample of the population. The results of the present study revealed that 0.05γ per 0.1 ml. of the PPD-s from the Japanese National Institute of Health was comparable in potency to 0.04γ per 0.1 ml. of the WHO PPD. The authors determined that the dosage of PPD preparation which was conveniently used in mass inoculation was that which produced skin reactions of the same size as those produced by 0.1 ml. of a 1:2,000 dilution of OT. There was, however, a slight difference in the readings of the size of reaction produced by the OT and the PPD preparations when the status of tuberculin allergy in the test population changed. However, this was not a great inconvenience in the analysis in mass surveys, and the authors concluded that 0.05γ per 0.1 ml. of the Japanese PPD-s was comparable to 0.1 ml. of the 1:2,000 OT currently used in Japan.—I. Tateno

Report of Two Antituberculosis Campaigns in Tunisia (in French). S. Bernard*. *Presse Med.*, June 18, 1960, 30: 1963–1964. (*Hopital Charles-Nicolle, Tunis, Tunisia)

An extensive antituberculosis campaign is under way for a population of 420,000 inhabitants in Tunisia. It is based on prophylactic measures and treatment. All persons up to the age of 20 years who are negative tuberculin reactors receive BCG vaccination. All persons older than 4 years are screened by roentgenography. The cases of minimal pulmonary tuberculosis are treated on an ambulatory basis and receive isoniazid only. Moderate and far advanced cases receive combined chemotherapy. They, too, are treated at home to a large extent, or hospitalized for 3 months and the treatment is then continued at home. Another program is conducted in Tunisia using isoniazid as

a prophylactic measure on a large scale. An evaluation of the results cannot be given at this time. It is expected that the results of these programs will be valuable to all countries with a high incidence of tuberculosis and insufficient facilities for treatment.—E. LYON

Oral BCG Vaccination. Leading Article. *Tubercle*, August, 1960, 41: 302–303.

Among the controversies which accompanied the introduction of BCG in England, little was heard of the merits of its oral administration. In Russia, where BCG is used on a very wide scale, the simplicity of oral vaccination is appreciated and its protective effect is believed to be at least as good as that with the intradermal method. Oral vaccination embraces points of practical and academic interest which repay study. It is now appreciated that tuberculin sensitivity of a similar order to that resulting from intradermal vaccination may be easily produced at all ages by increasing the oral dose far beyond that advocated by Calmette; and that these large doses of BCG by mouth are not associated with untoward effects. The presence of tuberculin sensitivity is generally considered to contraindicate intracutaneous vaccination, since untoward local reactions may occur. No adverse reactions are found, however, after the vaccination of tuberculin-sensitive persons by mouth. In consequence, prevaccination tuberculin tests are unnecessary when the vaccine is given in this manner; in Brazil, some 9 million persons have been vaccinated without prior tuberculin tests. There is some evidence to support the contention that vaccination of tuberculin-sensitive persons reduces their subsequent liability to tuberculosis. In those countries where the control of tuberculosis is less advanced than it is here, and where skilled medical and nursing services are in short supply, much might be gained by a properly controlled study of the oral method, which has the one great merit of simplicity.
—M. J. SMALL

BCG Vaccination of Premature Babies (in German). F. ECKARDT*. *Tuberkulosearzt*, June, 1960, 14: 333–345. (*Adlerstr. 35, Karlsruhe, Germany)

The tuberculin test was performed on 225 premature babies after BCG vaccination. The test was positive in more than 90 per cent of the cases in each weight group. There was no difference between premature and full-term babies with regard to the interval between the vaccination and the appearance of a positive reaction. No side effects were recorded. It was concluded that even the very young and immature organism was capable of immune reactions. Therefore, BCG vaccination could be given to premature babies 2 to 14 weeks after birth, thus reducing the isolation time before transferring the baby to a tuberculous home.
—J. HAAPANEN

Controlled Trial of BCG Vaccination in a School Population. G. W. COMSTOCK* and L. W. SHAW. *Pub Health Rep*, July, 1960, 75: 583–594. (*Tuberculosis Branch, Public Health Service, Washington, D. C.)

In April, 1947, a controlled trial of BCG vaccination was initiated in the school population of Muscogee County, Georgia. A total of 11,262 children had completed tests with 5 TU of PPD, and the nonreactors were tested with 100 TU. The nonreactors to both doses were divided into 2 similar groups; 1 group was vaccinated with BCG and the other left unvaccinated as a control. In the ensuing 12 years, 35 definite cases of tuberculosis were diagnosed among the study population. Three-fifths of the cases had clinically serious disease; a similar proportion was bacteriologically confirmed. In this area of low tuberculosis infection rates, it was not possible to demonstrate any benefit from BCG vaccination during 12 years of observation. It is suggested that in areas of low infection rates, which comprise most of the United States today, tuberculosis control programs among school populations might profitably be limited to periodic tuberculin testing surveys, with careful follow-up of reactors to a low dose.—E. DUNNER

The Incidence of Tuberculous Meningitis as an Indicator of the Efficacy of BCG Vaccination. V. VOJTEK*. *Tubercle*, August, 1960, 41: 272–275. (*State Children's Tuberc. Sanatorium, Sumperk, Czechoslovakia)

Compulsory BCG vaccination was introduced in Czechoslovakia in 1953, and an efficient system of notification of tuberculous meningitis in 1954. In 1954 there were 310 notifications of tuberculous meningitis in children younger than 15 years; in 1958 there were only 100. Notifications have been compared in vaccinated and unvaccinated children born from 1954 to 1958. There were 187 notifications. The notification rates were 13 to 15 times as great in the unvaccinated as in the vaccinated. It is realized that full and accurate assessment of the efficacy of BCG vaccination cannot be done by the crude method of comparing notification rates. Nevertheless, this comparison of notifications of tuberculous meningitis in vaccinated and unvaccinated children less than 5 years old does appear to show that the vaccine and methods of vaccination give good protection against the disease.—M. J. SMALL

Multiple-puncture Vaccination in the Newborn with Freeze-dried B.C.G. Vaccine. M. I. Griffiths*. *Brit Med J*, October 15, 1960, No. 5206: 1116–1119. (*U. of Manchester, England)

A total of 2,221 newborn infants were inoculated with British freeze-dried BCG vaccine by multiple-puncture vaccination between March, 1959, and April, 1960. Details of the technique are described and the significant factors are discussed. The results of multiple-puncture vaccination are compared with those of intradermal vaccination. It is suggested that, using the British freeze-dried vaccine, optimal results are obtained by firing 20 needles to a depth of 2 mm. through a vaccine concentration of 83 mg. per ml. It is hoped that by further improvement in technique the efficiency of multiple-puncture vaccination may be raised to the standard produced by intradermal vaccination (after author's summary).
—E. A. Riley

Detection of Pulmonary Tuberculosis by Sputum Survey. L. Erin*. *Tubercle*, October, 1960, 41: 363–366. (*Chest Clinic, Merthyr Tydfil, Glamorgan, Wales)

Although mass roentgenography is playing and will continue to play an important role in tuberculosis case finding, the yield per number examined is decreasing and the cost per case discovered is increasing. Furthermore, with present facilities only a small proportion of the general population of each clinic area can be examined, even at infrequent intervals. Therefore, the efficacy and cost of sputum testing as an additional technique which might be used by clinics to supplement the effect of mass roentgenography was investigated. A section of Mountain Ash (population 26,128), a mining town in the Aberdare Valley, Glamorgan, was chosen for the investigation. Of 1,982 specimens, 4 were found positive for tubercle bacilli. Approximately 2 sputum-positive cases were found per 1,000 persons examined, at a net cost of 25 pounds (100 pounds gross) per patient diagnosed. Suggestions are made as to how the technique could be improved, and the populations to which it could be most usefully applied.—M. J. Small

Tuberculosis in Ghana. A. B. P. W. Koch*. *Tubercle*, August, 1960, 41: 282–289. (*Tuberculosis Service Headquarters, Accra, Ghana)

In 1957, tuberculin testing was carried out in 7,400 persons of all age groups at 45 places throughout Ghana. This survey revealed that 55 per cent of the sample population were tuberculin positive; but more important was the fact that more than 25 per cent of the children up to 4 years of age showed positive reactions, and that after the age of 20 nearly ⅔ were tuberculin positive. The total number of cases of tuberculosis is estimated to be approximately 40,000. The proportional mortality for 1958 was 35 deaths from respiratory tuberculosis per 1,000 total deaths. Most disease was far advanced when first diagnosed. In 1959, 265 (about 31 per cent) of the new patients seen at the Korle Bu Clinic reported hemoptysis prior to first consultation. The main difficulty in organizing adequate treatment for as many patients as possible was the long traveling distance between the patient and the doctor. The first step in building up a tuberculosis service in Ghana, therefore, had to be to bring the clinic and treatment facilities as near as possible to the patient. A 5-year development plan was devised which provided regional tuberculosis centers in each of 6 regions. Altogether, about 14,000 cases of tuberculosis are at present under treatment or observation. In September, 1959, in cooperation with the Accra Health Committee and the Ministry of Education, a campaign was started to tuberculin test all Accra school children and to give BCG vaccination to the tuberculin-negative children. The campaign has just been completed and a total of about 35,000 children has been tested. Details are not yet available. The method of choice for the tuberculin test is now the Heaf test, which is easily and accurately performed even by junior nurses, is painless, and gives reliable results. For the test, OT diluted with the same quantity of glycerin plus 0.5 per cent of phenol is used. The final aim is to vaccinate every newborn, and to tuberculin test—and, if necessary, revaccinate—every child attending school, i.e., at about the age of 10 to 12. Chemoprophylaxis is not considered of value in a large-scale campaign in Ghana. A Ghana medical school is being planned and it has been proposed to establish the specialty of chest diseases within the medical faculty.—M. J. Small

Pulmonary Tuberculosis in Low Socioeconomic Classes (in Japanese). K. Shimamura*. *Clin Resp Organs*, September, 1960, 15: 600–604. (*National Kiyose Sanatorium, Tokyo, Japan)

Recent developments in chemo- and antimicrobial therapy and in surgical procedures in the treatment of pulmonary tuberculosis have reduced the number of deaths from this disease. These developments are an indication that pulmonary tuberculosis is a declining illness in the sense that few factors remain for future study. However, analysis of the socioeconomic status of tuberculous patients in general, and of those in this sanatorium, reveal that the status of tuberculosis has changed from that of a national disease to a disease of the poor people of the nation. Tuberculo-

sis was very prevalent in this nation and was at the top of the list of diseases with high mortality about 2 decades ago. Despite progress in treatment, widespread use of health-insurance systems by civil and public workers, and the optimistic indications described above, tuberculosis has remained a source of tragedy and a chronic incapacitating disease of poor people. This means that recent developments in the medical and social-work fields gave more remarkable benefits to people of the high-income group and less benefit to poor people, thus bringing the tuberculous illness of the low-income group to the attention of both professions. Greater efforts by medical and social workers for this group of people are urged.
—I. Tateno

The Pediatrician and Tuberculosis. Editorial. *J Pediat*, October, 1960, 57: 647–648.

A recent study by the U.S. Public Health Service has turned up some rather striking data on the risk of extrapulmonary complications of primary tuberculosis among tuberculin-positive infants and young children. This study was made in cooperation with 33 pediatric clinics throughout the United States, as well as in Mexico City, San Juan, and Toronto, and involved 2,750 subjects. It was clearly established that the risk of extrapulmonary complications is related to both age and extent of disease. Among infants less than 1 year of age who were tuberculin reactors, without roentgenographic evidence of tuberculosis, the risk of extrapulmonary involvement within 2 years after diagnosis was 1 in 30. If there was roentgenographic evidence of hilar and/or paratracheal involvement, the risk increased to 1 in 8. When parenchymal involvement was observed, the risk was even higher: 1 in 6. On the other hand, for children from 1 to 4 years of age, the risk was substantial (1 in 26) only when there was roentgenographic involvement. Fortunately, the study also established that the antituberculosis drug, isoniazid, can be expected to prevent 85 per cent of the progressive lesions that could otherwise be anticipated in infants and children in this age group. On the basis of these findings, the Public Health Service has recommended that all infants younger than 1 year who react to the tuberculin test, and all children from 1 to 4 years of age who have, in addition, roentgenographic evidence of primary tuberculosis, should be given 5 mg. of isoniazid per kg. of body weight daily for 1 year. On the basis of this new knowledge, tuberculin testing has become distinctly worth while, and isoniazid prophylaxis for reactors at high risk has become a necessity.—M. J. Small

Tuberculosis and Frontiers. Leading Article. Editorial. *Tubercle*, October, 1960, 41: 380–383.

The laws concerning tuberculosis and immigration are out of date. They sometimes appear to be used not to protect the population of the receiving country, the purpose for which they were devised, but to select the few from the many. This is administratively expedient, but it may be unjust. *The Lancet* suggested that the United Kingdom might offer to accept the "post-tuberculous" and their families on the understanding, in collaboration with Commonwealth Governments, that, if further investigation should confirm that the chest condition was satisfactory or if it was made satisfactory by treatment, they would be accepted as immigrants by one or another of these countries. This is an admirable suggestion and might be extended to include the "tuberculous," who could receive their treatment in this country on the same understanding with regard to their future emigration. Moreover, there seems no good reason, considering the small numbers likely to be involved, why Great Britain should not accept the so-called "incurable," as well, for permanent residence and care in England. In the future, no doubt, tuberculosis will have been reduced to the level of smallpox, with large areas of the world free or almost free from it. One may then require a certificate of a negative tuberculin test or recent BCG vaccination before crossing a frontier, even as a visitor. At present, however, the exclusion under all circumstances of those with roentgenographically manifest tuberculosis seems unjustified and, with regard to refugees, even inhumane.
—M. J. Small

Tuberculosis Undefeated. J. Crofton*. *Brit Med J*, September 3, 1960, No. 5200: 679–687. (*U. of Edinburgh, Scotland)

With 12 to 25 million infectious cases of pulmonary tuberculosis in the world, the disease is far from being defeated. Even in economically developed countries, the elimination of tuberculosis could be proceeding much more rapidly if present knowledge were more widely and more intensively applied. The main weapons for the defeat of tuberculosis are miniature roentgenography to detect cases, chemotherapy to cure them, and BCG to raise the individual's resistance to the disease. It is suggested that BCG has an important, if accessory, part to play in economically-developed countries, and might be even more useful in underdeveloped countries if the efficacy of the vaccine were kept constantly under review, both in the laboratory and in the field. The proper use of miniature roentgenography is reviewed. It is suggested this this weapon could be used with much

greater intelligence and effectiveness. Chemotherapy should be able to ensure the arrest of virtually all newly diagnosed cases of the disease, and there should be little or no relapse. Evidence for this is adduced. Methods of testing for drug resistance are at present chaotic. Failures of therapy are often due to the use of wrong combinations of drugs or to misleading drug-resistance tests. With good team work and proper treatment, the elimination of tuberculosis could be greatly accelerated. The Edinburgh figures for mortality and morbidity, although by no means perfect, have shown a dramatic change for the better over the last few years and now compare very favorably with other cities in the United Kingdom, Europe, and the United States. This is held to demonstrate the value of team work and of scrupulous chemotherapy in the elimination of the disease (after author's summary).—E. A. Riley

Tuberculosis: Report on the Reception of Ex-Patients in Respect of Re-employment and Superannuation, Life Assurance and Emigration. The Joint Tuberculosis Council of Great Britain. *Tubercle*, October, 1960, 41: 370–379.

This report deals with present-day attitudes toward ex-pulmonary tuberculosis patients with regard to employment, life insurance, and emigration. Inquiries were made of chest physicians throughout the country, and the more than 100 replies that were received are considered and discussed under the three main headings stated above. It is important to realize that these 3 problems are closely linked by the common factor of prognosis in pulmonary tuberculosis, especially after adequate treatment. If the promise of modern therapy is fulfilled, the long-term benefits to patients regarding employment and insurance could be almost the greatest and most important gain for their happiness and welfare. However, the confidence of the medical profession must be exerted to influence the authorities who control the financial aspects of insurance. Employment also often depends upon the acceptance of an ex-patient for the superannuation schemes of the firms, which are, of course, controlled by insurance companies. It is not the purpose of this report to summarize the modern theories of clinical treatment for pulmonary tuberculosis or to review the evidence which indicates that the disease can be considered curable if properly treated. The Council is, however, prepared to state its opinion that the whole outlook toward this disease has been so improved that in most cases the treated patient should not suffer undue handicap in employment and insurance matters. Correct treatment and adequate supervision can usually ensure an excellent prognosis. A plea is made, therefore, for a more generous attitude toward tuberculosis by insurance companies, and a request is made that some common policy be outlined. It is hoped that the publication of this report will draw attention to the need for a new attitude toward pulmonary tuberculosis so that advances in the clinical treatment of this disease will be rewarded, not only by clinical recovery, but by a greater measure of social and economic acceptance thereafter.
—M. J. Small

Air Pollution. Conference Report. *Public Health Rep*, December, 1960, 75: 1173–1189.

Summaries are presented of the 81 papers given at the Third Air Pollution Research Seminar held in New Orleans, Louisiana, on March 22–24, 1960.
—E. Dunner

Decline of the Tuberculosis Epidemic in Alaska. G. W. Comstock* and R. N. Philip. *Public Health Rep*, January, 1961, 76: 19–24. (*Public Health Service, Washington, D. C.)

Between 1949 and 1960, 3 tuberculin surveys were made in the Eskimo population living in the Yukon-Kuskokwim delta of Alaska. The results of tuberculin tests in children less than 3 years of age were used to obtain an estimate of the tuberculous infection rates. In 1949–1951, the average annual infection rate was 24.6 per cent; in 1957, it was 8.5 per cent; and in 1960, it was only 1.1 per cent. This dramatic decline appears to have resulted not only from improvements in social and economic conditions but, particularly, from a vigorous antituberculosis campaign with emphasis on case finding, isolation, and treatment.—E. Dunner

Pulmonary Fibrosis in a Worker Using an Aluminum Powder. J. W. Jordan*. *Brit J Industr Med*, January, 1961, 18: 21–23. (*Chest Clinic, Pontrfact, U. K.)

A 26-year-old woman was employed for 5 years in a fireworks factory and was exposed to finely powdered aluminum dust in the course of her work. She developed respiratory insufficiency, clubbing, tracheal deviation, and limited chest motion. Chest roentgenograms showed a fine infiltrate. During a 5-year follow-up period the patient developed a pneumothorax and arthritis in the hands, wrists, fingers, and shoulders. The latex fixation test was positive. At the present time, the clinical diagnosis of rheumatoid arthritis has been added to this patient's case. It is conceivable that the latter diagnosis could account for the entire clinical picture, but the author is more inclined to consider this patient's disease as a case of Caplan's syndrome due to aluminum inhalation (*see* Caplan, A.: *Thorax*, 1953, *8:* 29).—H. J. Simon

A Study of Aspergillus Spores in the Atmosphere of a Modern Mill. F. C. Stallybrass*. *Brit J Industr Med*, January, 1961, 18: 41–46. (*Royal South Hants Hosp., Southampton, England)

A study was made of the atmospheric pollution by Aspergillus spores in a large animal provender mill in which various grains are unloaded, stored, and processed, and a comparison was made with the degree of pollution of the urban atmosphere in the neighborhood. The catch-rate of the spores in the normal working atmosphere of the mill was found to be 30 times higher than that obtained in the urban atmosphere, and for this reason the health record of a group of operatives was considered with particular reference to the occurrence of pulmonary aspergillosis. Despite the risk of exposure within the mill, no evidence was found of an increased liability to develop the disease. The particular groups of aspergilli isolated by culture during this study have all been reported to have been associated with aspergillosis in man, and the relevant papers give evidence of a pathogenic rather than a saprophytic role. The study therefore provides quantitative data relating atmospheric spore content and the risk of pulmonary infection in man. In this study, of all of the grains handled in the mill, maize was associated with the highest degree of contamination.—H. J. Simon

Freeze-dried B.C.G. J. Lorber* and P. C. Menneer. *Arch Dis Child*, October, 1960, 35: 492–494. (*U. of Sheffield, England)

Freeze-dried BCG has been proved in the laboratory to be of uniform consistency with a viable bacterial count which, if kept in a cool place, persists unaltered in the dry state for a year or longer. It is safe and convenient to use. Suspensions of 0.1 ml. containing approximately $2–5 \times 10^6$ organisms per ml. were injected intradermally into more than 1,000 children. The result was a papule averaging 6 mm. in diameter. There was neither ulceration nor enlargement of the regional lymph nodes. Tuberculin conversion was demonstrated in virtually 100 per cent 6 to 8 weeks after vaccination, and a positive tuberculin reaction was maintained for at least 1 year after vaccination. In the case of others who were vaccinated with weaker vaccines, the proportion of positive reactors increased with time, indicating the probability that prolonged tuberculin sensitivity will be produced with the freeze-dried vaccine in current use.—J. Lorber

Bovine Infection in Extrapulmonary Tuberculosis in Humans (in Hungarian). I. Szabó* and N. Kertay. *Tuberkulozis*, July, 1960, 7: 198–199. (*Korányi Tbc. Intézet, Budapest, Hungary)

Extrapulmonary tuberculosis in 88 (35.4 per cent) of 248 patients was caused by bovine tubercle bacilli; 5 patients (2.1 per cent) had both human and bovine infections. In Hungary, 20 per cent of the cattle are tuberculin positive, and 5 to 6 per cent are positive for acid-fast bacilli as well.—Z. Virágh

Bronchitis—Sickness Absence in London Transport. C. J. Cornwall* and P. A. B. Raffle. *Brit J Industr Med*, January, 1961, 18: 24–32. (*London Transport Executive, England)

This study was based on the records of sickness absence of 4 days or longer attributed to bronchitis among nearly 60,000 London transport employees during the years 1952 to 1956. The figures support previous observations that there is a close relationship between the incidence of bronchitis and the occurrence of fog in any 1 year. The bronchitis experience of employees living and working in the northeastern sector of London is shown to be worse than in other areas of London. The experience of employees in the country belt around London, particularly in the southern sector, is better than in London itself. With the possible exception of transport conductors, the figures do not sugest that there are occupational factors in the transport industry which cause bronchitis (after authors' summary).—H. J. Simon

Bronchitis, Bronchiolitis, and Silicosis (in German). F. Nager*, F. Zenger, and J. R. Rüttner. *Schweiz Med Wschr*, November 26, 1960, 90: 1357–1364. (*Histopathologisches Institut, Zurich, Switzerland)

Microscopic examination of 100 patients with pneumoconiosis revealed that bronchitis and bronchiolitis were present in all cases and in all stages of pneumoconiosis, even in early stages. The bronchitic changes were more severe in these patients than in 50 control patients with pulmonary congestion or emphysema. Ectasis and stenosis of bronchioles caused by pneumoconiotic bronchiolitis deformans are of special significance. The deformities of the bronchioli caused by dusts play an essential part in the development of perifocal and obstructive emphysema. They largely account for the frequently observed discrepancy between the insignificant roentgenographic findings and the severe impairment of pulmonary function. Bronchiolitis deformans is one of the main causes for the continuation of bronchiolitis even after complete removal from dust exposure because it furthers retention and susceptibility to infection.
—J. Haapanen

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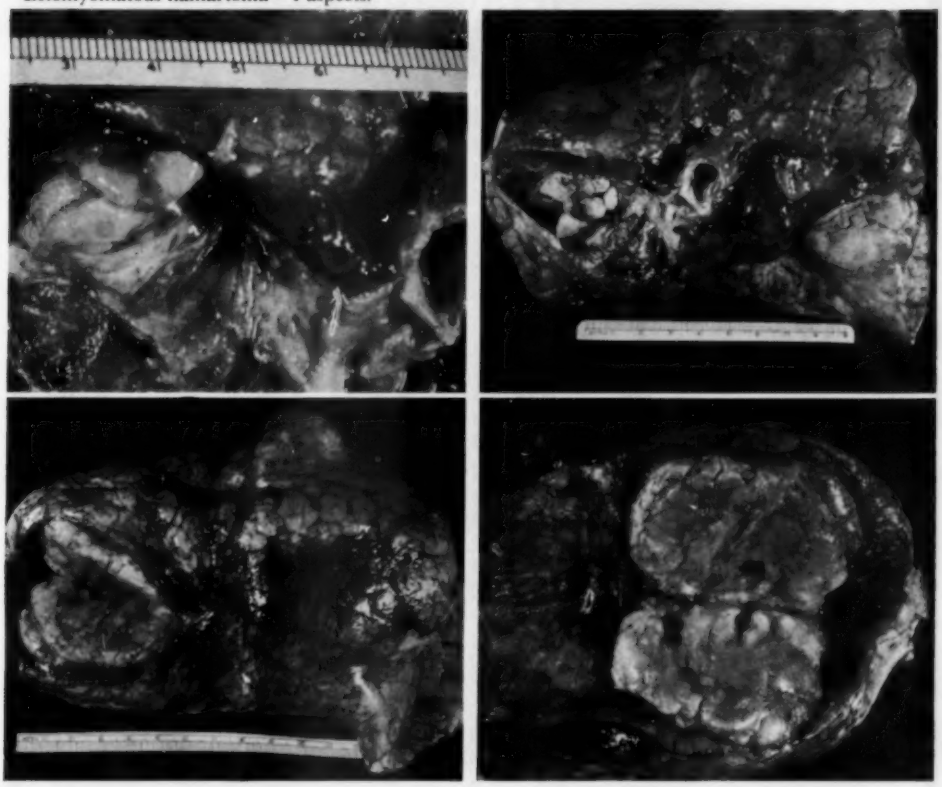

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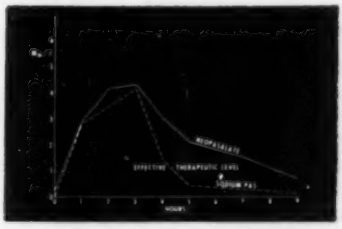

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Hospital administration	2	.45
Special research	1	.22
General public-health practice	10	2.29
Retired	3	.68
Not in practice	1	.22
No information	17	3.89
	350	80.19
Institutional subscribers	86	19.72
	436	99.91

AGE OF PHYSICIAN

	No.	%
No information	17	3.89
No age given	1	.22
30–34	10	2.29
35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
50–54	51	11.69
55–59	37	8.48
60–64	35	8.02
65 and over	36	8.25
	350	80.21
Institutional subscribers	86	19.72
	436	99.93

POPULATION

	No.	%
Under 10,000	56	12.84
10–25,000	39	8.94
25–50,000	44	10.10
50–100,000	38	8.71
100–200,000	32	7.33
200–500,000	48	11.00
500–1,000,000	39	8.94
Over 1,000,000	54	12.38
	350	80.24
Institutional subscribers	86	19.72
	436	99.96

BREAKDOWN OF INSTITUTIONAL SUBSCRIPTIONS

Federal Hospital	General Hospital	Special Hospital	Laboratory	Medical Schools	College University	Society Library	Population
3	2	5	2	0	1	0	Under 10,000
3	2	1	0	0	1	0	10–25,000
0	2	4	0	3	2	1	25–50,000
1	1	1	0	1	2	2	50–100,000
2	0	1	1	0	0	2	100–200,000
3	6	0	0	0	1	4	200–500,000
4	3	2	0	0	1	0	500–1,000,000
2	4	1	1	2	0	6	Over 1,000,000
18 4.12%	20 4.58%	15 3.44%	4 .91%	6 1.37%	8 1.82%	15 3.44%	86 19.72%

Percentages figured only to second decimal point

See inside front cover for subscription rate

DIFCO

Diagnostic Reagents for the Determination of

ANTISTREPTOLYSIN TITERS

Bacto-Streptolysin O Reagent is a standardized reagent recommended as an aid in the diagnosis of Rheumatic Fever, Glomerulonephritis and other diseases associated with Group A streptococcal infections.

SENSITIVITY TESTS

The significance of determining bacterial sensitivity is accentuated by the discovery of increasing numbers of antibiotics, sulfonamides, and other chemotherapeutic agents and the development of resistant organisms to one or more of these compounds. Sensitivity or resistance is easily determined with *Bacto-Sensitivity Disks and Bacto-Unidisks.*

HETEROPHILE ANTIBODIES

The differential diagnosis of Infectious Mononucleosis requires the use of standardized heterophile reagents: Bacto-Guinea Pig Kidney Antigen, Desiccated and Suspension, Bacto-Beef Cell Antigen, Desiccated and Suspension.

BLOOD COAGULATION

Prothrombin determinations require accurately prepared interstandardized reagents: Bacto-Thromboplastin, Bacto-Prothrombin 2 Stage Reagent, Bacto-Prothrombin Free Beef Plasma, Bacto-Prothrombin Free Rabbit Plasma, Bacto-AC Globulin, Bacto-Sodium Chloride 0.85%, Bacto-Sodium Oxalate Solution 0.1 Molar, Bacto-Calcium Chloride Solution 0.02 Molar.

LIVER DISEASE

Differential diagnosis of hepatic disturbances is aided by standardized reagents: Bacto-Cephalin Cholesterol, Antigen, Bacto-Thymol Turbidity Reagent, Bacto-Kingsbury Turbidity Standards, Bacto-Thromboplastin and other Prothrombin Reagents.

RENAL FUNCTION

Phenolsulfonphthalein Ampules, Difco is a carefully prepared injectable recommended for use in determining renal excretion rate.

HEMAGGLUTINATION OF RED BLOOD CELLS

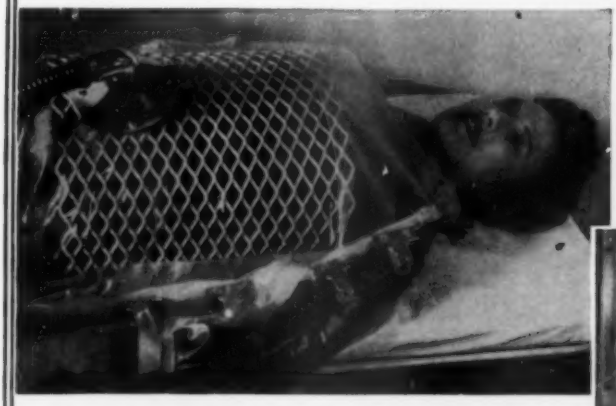
Accurately determined by the use of: Bacto-Trypsin 1% for Hemagglutination, Bacto-Hemagglutination Buffer, Trypsin, Difco, 1:250, Bacto-Bovine Albumin 30%, Bacto-Phytohemagglutinin

DIFCO LABORATORIES · DETROIT 1 MICHIGAN

patient-triggered

EMERSON
"Ucyclit-C"

body respirator for severe emphysema



Follows patient's breathing pattern (like an IPPB but without mask or mouthpiece). Drops slowly to atmospheric, after the peak of inspiration, to avoid air-trapping.

Please request Form 83-C

J. H. EMERSON CO., CAMBRIDGE 40, MASSACHUSETTS

SIMPLIFIES TUBERCULIN TESTING
STERNEEDLE* GUN

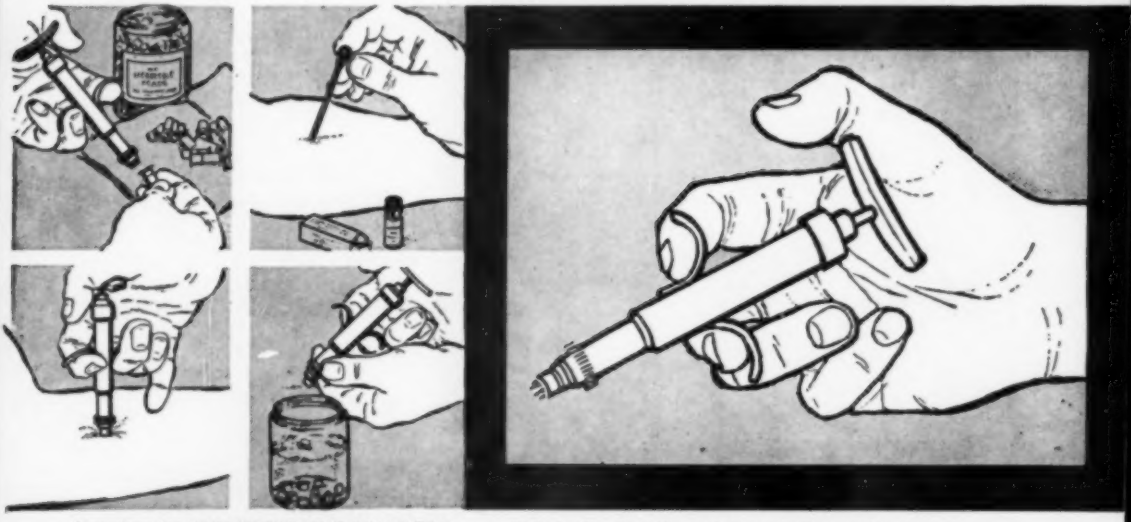
The Automatic TB Case-Finding Device for the Heaf TUBERCULIN TEST

The STERNEEDLE gun is an improved multiple puncture apparatus for rapid intradermal tuberculin testing. Based on the Heaf principle of multiple puncture, six needles contained within the STERNEEDLE cartridge mechanically and painlessly penetrate the skin through a previously applied film of concentrated PPD. Needle puncture and withdrawal is automatic.

ADVANTAGES...

- **Four (4) times faster than Mantoux.**
- **Faster and more reliable than Patch test.**
- **Performs up to 400 tests per hour.**
- **Detects reactors to 5 TU Mantoux and some 100 TU reactors.**
- **No dressing required.**
- **Accurate interpretation, without measurement. Averages 250 readings per hour.**
- **Each test is uniform—puncture of 1 mm. depth and needle withdrawal is automatic.**
- **No special skill required.**
- **Painless. Ideal for testing children.**
- **Eliminates hazardous and uncertain flame sterilization.**
- **Disposable needle cartridges ELIMINATE CROSS-INFECTION.**
- **Low cost STERNEEDLE cartridges may be resterilized.**

Note: CONCENTRATED TUBERCULIN PPD 'CONNAUGHT' is used for the STERNEEDLE test. Available in 1 cc vials (sufficient for as many as 200 tests).



Procedure for STERNEEDLE TUBERCULIN TEST:

Top, left... Slip needle cartridge on gun with plastic applicator. Remove applicator.

Top, right... Place a drop of tuberculin PPD on cleansed forearm.

Bottom, left... Place gun on drop of tuberculin PPD. Pressure and release of handle automatically triggers and retracts the needles.

Bottom, right: Disposable needle cartridges and applicators may be washed and sterilized for reuse, if desired.

Write for literature. Personal demonstration may be arranged.

TARGET POINT CASE FINDING

THE Panray CORP.

266 South Dean Street, Englewood, New Jersey

*Trademark. Pat. Pend.